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EFFECTS OF SYNGENEIC FRESH AND LIQUID PRESERVED RED BLOOD CELLS ON PRIMARY AND METASTATIC GROWTH OF THE LEWIS LUNG CARCINOMA IN MICE

BY

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ABSTRACT

This study examined the effects of transfusion of fresh and liquid preserved syngeneic red blood cells on the primary and metastatic growth of the Lewis Lung carcinoma in mice. Transfusions of nonviable syngeneic red blood cells enhanced the growth of metastatic tumors in four out of the seven experiments. The conflicting results observed may have been due to the differences in the virulence of the tumor cells associated with the number of tumor transfers from one mouse to another for the various experiments.

Mice were infused with syngeneic fresh or liquid-preserved red blood cells, or with saline. Lewis lung carcinoma cells were infused 4 hours after infusion. The mice were not sacrificed in three of the studies. In other studies, the mice were sacrificed at various intervals following the intravenous infusion of the Lewis lung carcinoma cells. The growth of the tumor was determined from the size of the primary tumor, the number of metastatic foci, and from the proliferation of the tumor cells determined by the accumulation of 125-I Iododeoxyuridine (IUDR). Mice transfused with liquid-preserved red blood cells exhibited a greater number of lung tumors larger than 1 mm in diameter and a significantly greater accumulation of 125-IUDR in the lung than mice transfused with fresh syngeneic red blood cells or with saline. Four experiments in mice sacrificed on days 15 through 20 showed no significant differences whether fresh syngeneic red blood cells or saline was infused.

Syngeneic fresh and liquid-preserved red blood cells had no effect on the growth of Lewis lung carcinoma tumor cells in the footpad of the mouse, or on metastases in the lung following amputation of the extremity subcutaneously inoculated with the Lewis lung carcinoma tumor cells into the footpad.

The virulence of the tumor cells, which was influenced by the number of times the tumor cells were transferred to the mouse, influenced survival time. There were significant correlations between the number of tumor foci and the weight of the lung and liver. The accumulation of 125-IUDR radioactivity was significantly correlated to the number of tumor cells infused and to the number of tumor foci and the weight of the lung and liver.

INTRODUCTION

Blood transfusions administered in cases of severe trauma and major surgical operations, although they may be necessary, may produce adverse effects on the recipient's immune status. Burrows and Tartter[1] found that survival times, free of recurrence of cancer rates, were significantly lower in transfused than in non-transfused patients following curative operations for primary colorectal cancer. Similarly, perioperative blood transfusions, administered during curative operations for colorectal cancer, breast cancer, lung cancer, renal cancer, and gastric cancer, have been associated with lower survival rates and shorter recurrent-free intervals.[2-8] There have also been reports of perioperative blood transfusions and postoperative infectious complications.[9] Still other studies have shown no adverse effects between blood transfusions and prognosis after cancer operations.[4,10-12] Pre-transplant blood transfusions have been administered since 1973 in an attempt to improve kidney graft survival,[13] and results have led to posttransfusion studies of immunosuppression. Studies in humans and animals have shown the nonspecific immunosuppressive effects of allogeneic blood transfusions.[14-19]

In this study in mice, we evaluated the effects of transfusions of syngeneic fresh and liquid-preserved red blood cells on the primary and metastatic growth of the Lewis lung carcinoma. During liquid storage of blood at 4 C, red blood cells are rendered nonviable, and these nonviable red blood cells are removed rapidly from the circulation by the

reticuloendothelial system following transfusion. The nonviable red blood cells may affect the immune system in a manner similar to that reported for inert particulate material such as silica and carrageenan, which inhibit the function of macrophages and the reticuloendothelial system and produce immunosuppressive effects that improve graft survival in animals.[20,21] Silica, carrageenan, and gold salts have been reported to enhance lung metastasis in animals.[22-24]

This study in the rodent was done to determine whether the immune function is affected by nonviable red blood cells in transfused fresh and liquid preserved syngeneic red blood cells.

MATERIALS AND METHODS

Animals: Six- to eight-week-old male B6C3HF1 mice (C57BL/6 female X C3H/H2 male) were purchased from Jackson Laboratory, Bar Harbor, ME; and B6C3HF1 male mice, 6-8 weeks old, were purchased from Charles River Breeding Laboratories, Wilmington, MA. The mice were obtained from the two different sources because the first laboratory was temporarily out of a supply of mice. All mice were housed in central animal quarters with controlled light and temperature. Food and water were provided ad libitum.

Preparation of Mouse Red Blood Cell Suspensions for 4 C Storage: The mice were anesthetized by an intraperitoneal injection of 3.6% chloral hydrate (0.1 ml/gram body weight). A 10 ml blood sample was collected in heparinized syringes by aortic puncture through a laparotomy incision (fresh blood). A 10 ml volume of blood was collected in a sterile glass tube containing 14.3 units of heparin per ml of blood and was stored at 4 C for 16 to 19 days (liquid preserved). Both the fresh and the liquid-preserved blood samples were washed five times with 0.9% sodium chloride and then resuspended to a hematocrit of 45%.

Preparation of Lewis Lung Cell Carcinoma: The Lewis lung carcinoma was maintained in B6C3HF1 mice by growth in the subcutaneous tissue. The potency of the virulence of the tumor cells increased as they were transferred from one mouse to another mouse in order to grow an adequate number.

The Lewis lung cell line was obtained from the American Type Tissue Culture Collections, Rockville, MD. The lung tumor

cells arrived at our laboratory in the frozen state where they were thawed in a 37 C water bath and then transferred to a 250 ml tissue culture flask (Falcon Flask, Becton-Dickinson). Twenty ml of Dulbecco's Modified Eagle high glucose medium (GIBCO, Grand Island, NY) were added to the cells; they were grown for one week and then centrifuged at 200 x g and washed in a fresh medium, and counted in a Coulter Counter. To establish the Lewis lung carcinoma in vivo, 10^6 cells were injected subcutaneously into the hind leg of each mouse. The remainder of the cells were placed in a flask with fresh medium and grown for two passages, then frozen in 10% DMSO in a -80 C mechanical freezer and stored in the gas phase of liquid nitrogen at -150 C.

The mice having subcutaneous tumors about 1 cm in diameter were anesthetized with 3.6% chloral hydrate per 0.1 ml/gm body weight. The tumor area was sterilized with Betadine and 70% alcohol. The mouse was placed on a sterile gauze field for removal of the tumor. Using sterile instruments, the overlying skin was removed from the tumor. Tumor tissue was removed, placed in a 60 mm petri dish with Dulbecco's medium, and transferred to a laminar flow hood. The tumor mass was minced with 4-inch scissors for 5 minutes, and the tumor pieces were aspirated into a 3 ml syringe and placed into a 15 ml tube (Falcon, Becton-Dickinson). The tumor pieces were permitted to settle, and the excess medium was removed. Five ml of 0.3% trypsin solution (Flow Laboratories, McLean, VA) was added, and the tumor cells were incubated at 37.5 C for 30 minutes. The tumor cells were washed once in the Dulbecco's medium, passed

through a 200 mesh stainless steel screen, then washed twice with the Dulbecco's medium, and resuspended to a concentration of 5 x 10^6 cells/ml. This procedure is referred to as Digestion Method 1. The viability of the tumor cells was determined by the method of Dankberg and Persidsky[25] using fluorescein diacetate and ethidium bromide. Using this protocol the cells were 90-95% viable, but with 10 to 25 per cent aggregates containing 3 to 10 cells per aggregate.

Digestion method 2 was used to prepare the tumor cells for the remaining experiments. In order to reduce the number of cell aggregates, the tumor was removed and minced, and washing was performed as previously described except that the enzymatic digestion was as follows: the minced tumor cells were transferred to a 15 ml tube; 6 ml of collagenase solution (1 mg/ml of collagenase, Sigma type IV) was added; and the cells were incubated in a 37.5 C water bath for 20 minutes with agitation. At the end of the incubation period, Dulbecco's medium was added, the sample was centrifuged at 200 g for 4 minutes, the pellet was resuspended with vortex mixing, and the sample was washed again with Dulbecco's medium. The pellet was dispersed with vortex mixing, 8 ml of 0.3% trypsin was added, and the suspension was incubated for 30 minutes at 37.5 C with agitation.

A solution of 0.02% EDTA in saline was added, and the tumor cell suspension was placed on wet ice at 4 C for 5 minutes. The tumor cell suspension was washed twice in saline containing 0.02% EDTA, centrifuged at 300 g for 4 minutes, and resuspended in 0.9%

saline. The tumor cells were counted, and the viability was measured using ethidium bromide (EB) and fluorescein diacetate (FDA). The tumor viability was between 85 and 95 percent, with less than 5 percent aggregates consisting of 5 cells or fewer.

125I-Iododeoxyuridine Uptake: The extent of metastatic growth was determined from measurements of the uptake of ^{125}I-Iododeoxyuridine (^{125}IUDR) by cellular DNA in lung, liver and spleen.[26] One day before the mice were sacrificed, each mouse was injected intraperitoneally with 26 ug of 5-Fluoro-2'-deoxyuridine (Sigma, St Louis, MO) in order to inhibit thymidine nucleotide synthesis. One hour later, 0.5 uCi of 5-{^{125}I}-Iodo-2'-deoxyuridine (Amersham International plc. 5 uCi/mg) was injected so that it would be incorporated into new DNA synthesis.

Twenty-four hours following injection of ^{125}IUDR, the mice were sacrificed, and the radioactivity in the lungs, liver and spleen were counted in a well-type gamma counter (Searle Analytic, Model 1185).

Dose Response of Lewis Lung Carcinoma in B6C3F1 Mice: In each experiment, mice were divided into four groups: each group consisted of ten mice. The mice were heated under a lamp in order to dilate the vein before the injection of tumor cells. In the first group, each mouse was infused with a total of 20 million tumor cells in 0.25 ml tumor cell suspension through the lateral tail vein. In the second group, 10 million tumor cells were infused into the mice. In the third group, 5 million tumor cells were infused into the mice. In the fourth group, 1 or 2 million tumor cells were infused into the mice. In some

experiments, we recorded the survival time and the number of tumor foci in the lung, liver, spleen, heart, and kidney following the tumor cell infusions. In other experiments, the mice were sacrificed after 9 or 12 days, the uptake of 125IUDR by the growing tumors was measured in the organs, and the number of tumor foci was recorded.

Mouse Survival Following Red Blood Cell Transfusion and Intravenous Tumor Cell Injection: To study the effects of syngeneic fresh red blood cells and liquid-preserved red blood cells (RBC) or saline on the growth of Lewis lung tumor, the following protocol was carried out:

In each experiment, the mice were divided into three groups. In the first group, each mouse was infused with 0.9% sodium chloride (saline); in the second group, each mouse was infused with a fresh red cell suspension (fresh-RBC); in the third group, each mouse was infused with a liquid-preserved red cell suspension (stored-RBC). All injections were given in the retroorbital venous plexus under chloral hydrate anesthesia. Approximately 4 hours after injection, each mouse was injected with 0.25 ml of a Lewis lung carcinoma cell suspension containing 5×10^6 cells in the lateral tail vein. The survival of the mice was observed for 80 days.

Growth of Metastases Following Red Blood Cell Transfusion and Intravenous Tumor Cell Injection: The mice were injected with fresh-RBC, stored-RBC, or saline; 4 hours later, the tumor cell suspension was injected. The mice were sacrificed 15, 16, 20, 23, or 24 days following tumor cell injection. The number of

tumor foci in the lungs and livers was counted macroscopically. The diameter of each lesion was determined with the use of a digital readout micrometer (Mitutogo Corporation, Japan).

Growth of Primary Tumor Following Subcutaneous injection of Lewis Lung Carcinoma Cells in the foot pad of the mouse: Each mouse was inoculated with a 0.25 ml of Lewis lung carcinoma cell suspension containing 2×10^6 cells/ml in the left hind footpad under chloral hydrate anesthesia. Each mouse received four intravenous infusions of 0.25 ml of fresh-RBC, liquid-preserved-RBC, or saline in the lateral tail vein on the day prior to the subcutaneous inoculation of the tumor cells and again on days 4, 8, and 12 following inoculation. The size of the primary tumor was measured, and the tumor volume (V cm^3) was estimated from major axis (A cm) and minor axis (B cm) as follows: $V = AB^2.^{27}$

Growth of Spontaneous Metastases Following Removal of the Primary Tumor: Each mouse was inoculated with a tumor cell suspension in the left footpad. Twenty-one days later, the primary tumor was removed by amputating the left leg below the knee under chloral hydrate anesthesia. Approximately ten minutes following amputation, each mouse was infused with 0.25 ml of fresh-RBC, liquid-preserved-RBC or saline in the retroorbital venous plexus. The mice were sacrificed 8 to 10 days following amputation. The number of lung metastases was counted macroscopically, and the ^{125}IUDR uptake in the lungs was measured.

Bacterial Culture: All the red blood cell suspensions were cultured in aerobic and anaerobic broth and on blood agar plates

immediately before infusion. Tumor cell suspensions were cultured on agar plates.

Statistical Analyses: The significance of the differences in the number of metastases, the ^{125}IUDR uptake, and the volume of primary tumors among the three groups was determined by the analysis of variance, and the least-square means were used for multiple paired comparisons. The log rank test was used to test the significance of differences in the survival rate.

RESULTS

Survival of Mice Following Intravenous Infusion of Lewis Lung Carcinoma: As shown in Figure 1 and Table 1, survival was significantly shorter in the mice transfused with stored red blood cells than in those transfused with fresh red blood cells or saline before the intravenous infusion of the Lewis lung carcinoma cell suspension ($p<0.0001$). The tumor cell suspension was prepared according to Digestion Method 1 using trypsin alone, and the tumor cells were sterile for bacterial contamination. The mice transfused with fresh red blood cells exhibited significantly lower mean survival time than the mice transfused with saline ($p<0.001$). The mean survival time in the mice transfused with stored red blood cells was 20.0 ± 2.4 days, and that in the mice transfused with fresh red blood cells was 25.9 ± 3.9 days. Three of the 20 mice in the saline group survived more than 80 days. At the time of death, lung metastases were observed in 18 of the 20 mice infused with stored red blood cells, and liver metastases were observed (Table 2). Seven of the 20 mice transfused with fresh red blood cells (35%) had liver metastases, and 13 mice (65%) had lung metastases. Seven of the 17 mice transfused with saline (35%) had lung metastases, 7 (35%) had liver metastases, and 3 (17%) had other tumors.

The experiments were repeated using tumor digestion method 2 using collagenase and trypsin to isolate the tumor cells. The tumor cells isolated from the in vivo grown tumor were sterile for bacterial contamination. The viability of the tumor cells

was between 85 and 95%, with less than 5% aggregates that consisted of 5 cells or less. Figure 2 and Table 1 show results of the first experiment using Method 2 in which 5 X 10^6 tumor cells were injected. The mice transfused with 0.25 ml of syngeneic liquid-preserved red blood cells with a hematocrit value of 40% had a mean survival time of 13.7 days compared to 17.5 days for mice transfused with fresh red blood cells, and 17.1 days for mice transfused with saline. The mean survival time of mice transfused with fresh red blood cells or saline was significantly longer than that for mice transfused with liquid-preserved red blood cells ($p=0.017$).

Likewise, in mice infused with 2 x 10^6 Lewis lung tumor cells, mean survival times were significantly different among the groups (Figure 3 and Table 1). The mean survival time for mice transfused with stored red blood cells was 19.2 days, for mice transfused with fresh red blood cells mean survival was 21.1 days, and the mean survival time for mice transfused with saline was 24.6 days.

In two subsequent experiments, no significant differences in mean survival times were noted whether the mice were transfused with fresh red blood cells, liquid-preserved red blood cells, or saline, before infusion of 2 X 10^6 tumor cells (Figures 4 and 5 and Table 1). These findings suggest the possibility that the tumor cells had become more virulent due to the number of times the tumor was transferred from one mouse to another mouse.

Two additional studies were done in mice intravenously infused with 1 X 10^6 tumor cells isolated by Digestion Method 2

to assess the effects of fresh red blood cells, liquid-preserved red blood cells, and saline.

The mice transfused with stored red blood cells died sooner than mice transfused with saline or fresh red blood cells prior to the intravenous infusion of 1 X 10^6 tumor cells ($p=0.009$). In the seventh study, mice transfused with stored red blood cells or saline exhibited shorter survivals than mice transfused with fresh red blood cells (Figure 7 and Table 1).

Growth of Metastases Following Intravenous Tumor Cell Infusion: Syngeneic fresh and liquid-preserved red blood cells, and saline, transfused following the intravenous infusion of Lewis lung carcinoma cells were evaluated to determine their effects on the number of metastatic foci and 125-IUDR incorporation in the tissue of mice sacrificed from 15 to 24 days after infusion of tumor cells.

On days 15 and 16, the mice transfused with liquid-preserved red blood cells were found to have a significantly greater number of tumors, and more that were larger than 1 mm, than in mice transfused with fresh red blood cells or saline (Table 3). The uptake of 125-IUDR in the lungs and liver was significantly greater in mice transfused with liquid-preserved red blood cells than in mice transfused with fresh red blood cells or saline (Table 3).

In experiments done to determine the effect of fresh red blood cells on the metastases of Lewis lung carcinoma, mice were transfused with fresh red blood cells or saline, and 4 hours later infused intravenously with 1 million tumor cells. The mice

were sacrificed on days 20, 23 and 24. Although the number of tumors in the lung on day 24 was significantly greater in the mice transfused with fresh red blood cells than in mice transfused with saline, there were no significant differences between the two groups on days 20 through 23 (Table 4). On day 23, the mice transfused with fresh red blood cells exhibited a greater number of liver tumors and greater 125-IUDR uptake than mice transfused with saline.

Table 5 reports three experiments in which tumor cells prepared by Digestion Method 2 were infused. These mice were not sacrificed, but died spontaneously. The survival time and number of metastatic foci found in the lungs, liver, spleen, heart and kidneys were measured. In all three experiments, there were similarly large numbers of metastatic foci found in the lungs of the mice transfused with liquid-preserved red cells and saline, but in two experiments survival times were shorter in the mice transfused with liquid-preserved red cells.

Growth of Primary Tumor Following Footpad Inoculation of Tumor Cells: In two experiments in which the mice were transfused with fresh or liquid-preserved red cells or saline before and after receiving footpad inoculations with tumor cells prepared by Digestion Method 1, the growth of the primary footpad tumor was investigated. In one experiment, the size of the footpad tumor was significantly larger in the mice transfused with fresh or liquid-preserved red cells than in mice transfused with saline on days 22 to 26 post-inoculation (Table 6). However, no differences were observed in the second experiment.

Growth of Metastases Following Removal of the Primary Footpad Tumor: The tumor was prepared by Digestion Method 1. The mice were transfused with syngeneic fresh or liquid-preserved red blood cells, or saline the day prior to inoculation of the tumor cells into the footpad, and on days 4, 8 and 12 following inoculation. The number of lung metastases and the ^{125}IUDR uptake in the lung were slightly greater in the mice transfused with liquid-preserved red blood cells than in the mice transfused with saline 8 days following amputation of the extremity inoculated with tumor cells (Table 7). Nine days following amputation, the total number of lung metastases and the number of metastatic foci larger than 1 mm in size were significantly greater in the mice transfused with liquid-preserved red blood cells than in the mice transfused with fresh red blood cells or saline, although no differences were observed in 125-IUDR uptake. Eleven days following amputation, the number of lung metastases was significantly greater and 125-IUDR was slightly greater in the mice transfused with liquid-preserved red blood cells than in the mice transfused with fresh red blood cells (Table 7).

Results of Tumor Cell Growth and Metastases of Lewis Lung Tumor Prepared by Digestion Method 2: In these experiments, the Lewis lung carcinoma cell suspension was prepared by the collagenase and trypsin digestion method referred to as Digestion Method 2.

Tumor Cell Dose and Survival Time in Mice: In 4 experiments to evaluate the effects of the dose of Lewis lung tumor on mouse survival, the log rank test of survival time showed a significant reduction in survival time associated with increased dosages of

tumor cells (Table 8). A total of 130 mice were infused with 1, 2, 5, 10 and 20 million cells per mouse. The mean survival ranged from 23 days for mice receiving 1 million cells to 11.3 days for mice receiving 20 million cells (Tables 8-12). The mice survived for 18.5 days when 2 x 10^6 tumor cells were infused and 12.3 days when 20 million cells were intravenously infused.

Using Digestion Method 1, up to 25% of the cells injected were clumped; clumps with as many as 5-10 cells per clump were observed. We estimated that as many as 2 million tumor cells were transfused in these earlier experiments, so we used this number for our lowest dose of cells in the dose response experiments.

Tumor Cell Dose Response as Measured by 125IUDR Uptake and Number of Metastatic Foci: In order to determine whether there was a correlation between metastatic foci and the number of tumor cells administered, cell proliferation was assessed in four experiments from 125-IUDR incorporation into DNA. In one experiment we planned to sacrifice the mice on day 12, but several mice died before day 12, so in subsequent experiments the mice were sacrificed on day 9. The number of infused tumor cells correlated significantly with the number of metastatic foci in the lung (r=0.914) and the weight of the lung and liver (r=0.884) (Table 13). The number of infused tumor cells also correlated with the 125-I radioactivity in the lung (r=0.828), and the uptake of 125-I correlated with the number of foci and the weight of the lung and liver (r=0.884). The data for these experiments are reported in Tables 14-18 and Figures 8-13.

DISCUSSION

In four mouse experiments, transfusions of syngeneic liquid-preserved red blood cells were associated with greater enhancement of growth of metastatic tumors and greater reductions in survival time following intravenous injection of the Lewis lung carcinoma cell suspension than transfusions of either fresh red blood cells or saline, suggesting that the liquid-preserved red blood cells produced immunosuppression. However, in three other experiments, there were no detectable effects on immune suppression associated with liquid-preserved red blood cells, fresh red blood cells, or saline.

The Lewis lung carcinoma cell line was grown *in vivo* for all experiments. In the early experiments there was variability due to incomplete digestion of the solid tumor, resulting in large clumps of three to ten cells per clump, with 10 to 25% of the cells infused being clumps of cells. In the later experiments the tumor cells killed the mice faster with shorter mean survival times. It appears from these results that a more virulent strain of tumor cells was selected by the *in vivo* growth of the tumor cells as indicated by the decreased survival time.

Liquid preserved syngeneic red blood cells were associated with increased growth of metastatic tumors and reduced survival rates following intravenous injection of the Lewis lung carcinoma cell suspension. Metastases increased following removal of the primary tumor, although the growth of the primary tumor was not consistent. The average 24-hour posttransfusion survival of mouse red blood cells stored at 4 C for 15 days was 41%, and the

24-hour posttransfusion survival of mouse red blood cells stored at 4 C for 24 days was 30%.[28]

The average 24-hour posttransfusion survival of fresh mouse red blood cells was 95%.[28] The number of nonviable red cells injected into each mouse was estimated to be approximately 1.6 X 10^9 in the stored-RBC group and 1.4 X 10^8 in the fresh-RBC group when calculated using average red blood cell counts and hematocrit of the mouse. Nonviable red blood cells in the fresh red cell suspensions, although small in number, might have had some influence on the macrophage function. The nonviable liquid-preserved red blood cells were sequestered mainly in the liver, spleen, and bone marrow; small uptake was observed in the lung and kidney.[28] It has been known that senescent or injured red cells are digested enzymatically within the macrophages following phagocytosis.[29] Ingestion of nonviable red blood cells by macrophages may adversely affect the function of the macrophage to remove cancer cells from the circulation.

Most tumor cells in the bloodstream are arrested in the microvasculature and killed immediately.[30] Mechanisms of killing include mechanical trauma, oxygen toxicity, inflammatory responses mediated by polymorphonuclear neutrophils, natural killer cells, and specific immune responses mediated by macrophages and lymphocytes.[30,31] Deterioration of macrophage function may reduce both nonspecific and specific immune responses to tumor cells.

The survival and growth of metastases following intravenous tumor cell injection were adversely affected by the transfusion

of fresh syngeneic red blood cells, but not to as great an extent as that observed with the transfusion of liquid-preserved red cells. Most of the mice infused with liquid-preserved red blood cells died with lung tumors, whereas mice transfused with fresh red blood cells or saline exhibited both lung and liver metastases at the time of death.

There have been few reported studies on the effects of syngeneic blood transfusions on the tumor growth or immune function. However, studies in rodents have shown that allogeneic blood transfusions induce immunosuppression and increase tumor growth.[32-35] Judson et al[36] found that survival rates in mice injected with mammary tumor cells in the inguinal mammary pad were lower in mice transfused with allogeneic blood than in non-transfused mice. Waymack et al[37] reported that allogeneic and syngeneic nonviable red blood cells induced suppressor activity in macrophages.

It is difficult to apply our data from the study of mouse lung metastases to humans because the intravenous injection of the tumor cell suspension is not physiological, and tumor growth was found not only in the lung but also in the liver, a site where spontaneous metastases from subcutaneously inoculated Lewis lung carcinoma is rarely observed. Neither the fresh nor the liquid-preserved red blood cells increased the number of metastases following the removal of the primary tumor of the footpad. Several nonspecific immunosuppressive effects of allogeneic blood transfusions have been reported, e.g., suppression of cellular immunity, as indicated by a reduced

response to mitogenic and antigenic stimulation,[14] decreased natural killer cell activity,[15] increased suppressor cell activity of both T-cells and monocytes,[16,17] and increased production of immunosuppressive prostaglandin E by macrophages.[18] The effects of blood transfusions on the augmentation of metastases following operations for cancer in our study may have been due to the percentage of nonviable red blood cells transfused.

LISTING OF TABLES

Mice transfused with fresh syngeneic red blood cells, liquid-preserved syngeneic red blood cells or saline, followed by infusion of tumor cells

1. Comparison of digestion methods 1 and 2 and mean survival times of the mice.
2. Number of tumors at time of death; tumor cells isolated by digestion method 1.
3. Number of tumors and 125IUDR uptake by time in the mice sacrificed on the 15th and 16th day following infusion of the tumor cells; tumor cells isolated by digestion method 1.
4. Number of tumors and 125IUDR uptake by time in mice sacrificed on the 20th to the 24th day following infusion of the tumor cells; tumor cells isolated by digestion method 1.
5. Size of primary tumor in the footpad at time of sacrifice; tumor cells isolated by digestion method 1.
6. Number of tumors and 125IUDR concentration after removal of primary tumor at time of sacrifice; tumor cells isolated by digestion method 1.
7. Mean number of tumors at time of death; tumor cells isolated by digestion method 2.

Nontransfused mice infused with increasing numbers of tumor cells digestion method 2

8. Comparison of mean survival times of the mice.
9. Survival time and number of tumor foci in exp. 1
10. Survival time and number of tumor foci in exp. 2
11. Survival time and number of tumor foci in exp. 3
12. Survival time and number of tumor foci in exp. 4
13. Correlation between dose of tumor cells and: 125IUDR uptake and number of tumor foci at time of sacrifice
14. Mean 125IUDR uptake at time of sacrifice
15. Mean number of tumor foci at time of sacrifice

16. 125IUDR uptake and number of tumor foçi at time of sacrifice in exp. 1

17. 125IUDR uptake and number of tumor foci at time of sacrifice in exp. 2

18. 125IUDR uptake and number of tumor foci at time of sacrifice in exp. 3

TABLE 1.

SURVIVAL TIME FOLLOWING TRANSFUSION WITH SYNGENEIC FRESH RED BLOOD CELLS OR LIQUID PRESERVED RED BLOOD CELLS OR SALINE FOLLLOWED 4 HOURS LATER BY THE INFUSION OF LEWIS LUNG TUMOR CELLS

	Log Rank Test	Mean Survival Time (days)			Number of in vivo transfers of tumor cells prior to infusion
	p value	saline	Fresh RBC	Stored RBC	
1. Digestion Method 1 n=60, 20 per tx group $1x10^6$ tumor cells infused	(<0.05)	~30 (3 did not die)	25.9	20*	8
2. Digestion Method 2 n=30, 10 per tx group $5x10^6$ tumor cells infused	(0.017)	17.1	17.5	13.7*	10
3. Digestion Method 2 n=30, 10 per tx group $2x10^6$ tumor cells infused	(0.019)	24.6+	21.1+	19.2+	12
4. Digestion Method 2 n=30, 10 per tx group $2x10^6$ tumor cells infused	(NS)	16.3	16.2	16.6	22
5. Digestion Method 2 n=30, 10 per tx group $2x10^6$ tumor cells infused	(NS)	16.9	16.3	16.3	26
6. Digestion Method 2 n=30, 10 per tx group $1x10^6$ tumor cells infused	(0.009)	17.7	18.4	15.9*	16
7. Digestion Method 2 n=30, 10 per tx group $1x10^6$ tumor cells infused	(0.003)	20.8	24.5^	21.4	25

Significant pairwise comparisons between groups; Log rank test, $p<0.05$

* between liquid preserved RBC and both saline and fresh RBC.

\+ between all three groups.

^ between fresh RBC and both saline and liquid preserved RBC.

TABLE 2.

NUMBER OF MICE WITH METASTASES FOLLOWING INFUSION OF 1 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 1 AT THE TIME OF THE DEATH OF THE MICE

Transfusion Group	n	with lung tumors	with liver tumors	with lung & liver tumors	with other tumors
Saline	17	7	7	7	3
Fresh RBC	20	13	7	7	0
Stored RBC	20	18	2	2	0

TABLE 3.

EFFECT OF SYNGENEIC FRESH AND LIQUID-STORED RED BLOOD CELL AND SALINE TRANSFUSIONS ON LUNG AND LIVER METASTASES 15 AND 16 DAYS FOLLOWING INFUSION OF 1 X 10^6 TUMOR CELLS DIGESTED BY METHOD 1. THE MICE WERE SACRIFICED

Transfusion Group	n	Number of Tumors Greater Than 1 mm in Diam.	Total	125IUDR Uptake (cpm)
LUNG				
DAY 15				
Saline	10	0	1.2±1.4	191±69
Fresh RBC	10	0.3±0.5	4.4±9.1	213±111
Stored RBC	9	3.3±3.1*+	7.0±6.0	650±386*+
DAY 16				
Saline	6	1.0±0.6	4.8±5.0	268±182
Fresh RBC	6	1.0±0.6	4.2±2.3	256±203
Stored RBC	6	6.3±3.8*+	18±5.5*+	1293±759*+
LIVER				
DAY 15				
Saline	10		0.3±0.5	1454±326
Fresh RBC	10		1.5±1.1	1934±789
Stored RBC	9		3.1±6.4	2309±787*
DAY 16				
Saline	6		1.2±1.6	1148±644
Fresh RBC	6		3.0±2.3	2009±1448
Stored RBC	6		12.8±11.5*+	7563±8503

*significantly higher than the saline group ($p<0.05$)

+significantly higher than the fresh RBC group ($p<0.05$)

TABLE 4.

EFFECT OF SYNGENEIC FRESH RED BLOOD CELL AND SALINE TRANSFUSIONS ON LUNG AND LIVER METASTASES 20 TO 24 DAYS FOLLOWING INFUSION OF 1 X 10^6 TUMOR CELLS DIGESTED BY METHOD 1. THE MICE WERE SACRIFICED

Transfusion Group	n	Number of tumors Greater than 1 mm in Diam.	Total	125IUDR Uptake (cpm)
LUNG				
DAY 20				
Saline	10	0.4±0.7	9.8±13.8	426±465
Fresh RBC	10	1.6+1.8	10.0+9.1	394+275
DAY 23				
Saline	8	1.1±1.1	9.6±7.7	553±422
Fresh RBC	9	5.9±10.3	37.2±46	1550±1865
DAY 24				
Saline	10	0.6±1.0	4.2±4.7	526±485
Fresh RBC	9	4.9±5.8	48.8±46.3*	1766±2204
LIVER				
DAY 20				
Saline	10		1.4±1.0	2228±1456
Fresh RBC	10		1.9±1.1	3564±3278
DAY 23				
Saline	8		3.4±1.4	12852±10478
Fresh RBC	9		6.6±2.1*	29832±12933*
DAY 24				
Saline	10		0.9±1.4	2516±2073
Fresh RBC	9		1.4±1.9	3878±2204

*significantly higher than the saline group ($p<0.05$)

TABLE 5

NUMBER OF TUMOR METASTASES IN LUNG, LIVER, SPLEEN, HEART AND KIDNEY OBSERVED AFTER DEATH IN MICE TRANSFUSED WITH SYNGENEIC FRESH AND LIQUID STORED RED BLOOD CELLS OR SALINE, FOLLOWED 4 HOURS LATER BY THE INFUSION OF LEWIS LUNG CARCINOMA CELLS PROCESSED BY DIGESTION METHOD 2

TRANSFUSION GROUP	n	MEAN DAYS SURVIVED	MEAN NUMBER OF TUMORS LUNG	LIVER	SPLEEN	HEART	KIDNEY
EXPERIMENT 1							
Saline	9	17	30	17	--	--	--
Fresh RBC	8	18	36	13	--	--	--
Stored RBC	5	14	36	23	--	--	--
EXPERIMENT 2							
Saline	10	18	74	0	0	0	0
Fresh RBC	10	18	50	0	0	0	0
Stored RBC	10	16	73	0	0	0	0
EXPERIMENT 3							
Saline	10	21	48	10	2	0	2
Fresh RBC	10	25	30	8	3	0	1
Stored RBC	10	21	53	13	2	0	2

TABLE 6.

EFFECT OF SYNGENEIC FRESH AND LIQUID STORED RED BLOOD CELL AND SALINE TRANSFUSIONS ON GROWTH OF PRIMARY FOOTPAD TUMORS 15 TO 26 DAYS FOLLOWING SUBCUTANEOUS INJECTION OF TUMOR CELLS DIGESTED BY METHOD 1 INTO THE FOOTPAD

Transfusion group	n		Volume of tumor (cm^3)					
			15day	18day	20day	22day	24day	26day
EXPERIMENT 1								
Saline	10	MEAN	0.11	0.20	0.36	0.63	0.99	1.37
		SD	0.03	0.04	0.10	0.14	0.24	0.32
Fresh RBC	10	MEAN	0.14	0.27	0.53	0.95*	1.54*	2.14*
		SD	0.03	0.10	0.24	0.29	0.39	0.45
Stored RBC	9	MEAN	0.13	0.26	0.53	1.05*	1.70*	2.46*
		SD	0.03	0.08	0.02	0.36	0.56	0.37
EXPERIMENT 2								
Saline	10	MEAN	0.14	0.32	0.65	1.11	1.78	2.67
		SD	0.05	0.14	0.26	0.40	0.60	0.84
Fresh RBC	10	MEAN	0.15	0.34	0.64	1.08	1.77	2.52
		SD	0.03	0.08	0.21	0.30	0.37	0.40
Stored RBC	10	MEAN	0.16	0.36	0.66	1.13	1.66	2.46
		SD	0.04	0.09	0.17	0.25	0.36	0.47

*significantly higher than the saline group ($p<0.05$)

TABLE 7.

EFFECT OF FRESH AND LIQUID STORED RED BLOOD CELL AND SALINE TRANSFUSIONS ON LUNG METASTASES FOLLOWING REMOVAL OF PRIMARY FOOTPAD TUMOR 8 TO 11 DAYS FOLLOWING THE AMPUTATION

DIGESTION METHOD 1, (mean, sd)

Transfusion Group	n	Number of lung tumors Greater than 1 mm in Diam.	Total	125IUDR Uptake (cpm)
8 DAYS FOLLOWING AMPUTATION				
Saline	9	2.2±2.8	17.7±6.9	864±499
Fresh RBC	10	3.9±4.2	24.2±11.5	1117±609
Stored RBC	10	5.6±4.3	28.3±14.8	1530±1318
9 DAYS FOLLOWING AMPUTATION				
Saline	9	13.1±11.5	56.7±21.2	2066±1172
Fresh RBC	10	14.8±9.9	33.4±14.1	1791±1414
Stored RBC	9	17.9±11.4	79.2±23.5*+	1872±1844
11 DAYS FOLLOWING AMPUTATION				
Saline	10	16.1±5.9	30.3±6.2	4046±1979
Stored RBC	10	22.3±5.5+	44.9±12.2+	6125±1581

*significantly higher than the saline group ($p<0.05$)

+significantly higher than the fresh RBC group ($p<0.05$)

TABLE 8

MEAN SURVIVAL TIME OF MICE IN DAYS AFTER THE INTRAVENOUS INFUSION OF 1, 2, 4, 10, AND 20 X 10^6 LEWIS LUNG CARCINOMA CELLS IN 4 STUDIES

		Survival Time (days)			
Tumor cells infused (millions):		2	5	10	20
STUDY 1	Mean:	21.4	16.5	-	13.1
	SD	3.1	2.6		1.7
	N	8	8		7

Log rank test, survival: p value
All dosages: 0.0001
2 vs 5: 0.004
5 vs 20: 0.014

		2	5	10	20
Study 2	Mean:	18.5	16.4	13.0	12.3
	SD	1.5	2.5	1.2	1.4
	N	11	10	9	10

Log rank test, survival: p value
All dosages: 0.0001
2 vs 5: 0.0308
5 vs 10: 0.0005
10 vs 20: 0.273 (not significant, p>0.05)

		2	5	10	20
Study 3	Mean:	18.9	16.0	13.6	11.3
	SD	2.6	1.3	1.8	1.1
	N	10	9	9	9

Log rank test, survival: p value
All dosages: 0.0001
2 vs 5: 0.0085
5 vs 10: 0.0082
10 vs 20: 0.0073

		1	5	10	20
Study 4	Mean:	22.9	18.0	18.2	-
	SD	3.6	2.2	2.3	
	N	10	10	10	

Log rank test, survival: p value
All dosages: 0.0001
1 vs 5: 0.0002
5 vs 10: 0.85 (not significant)
1 vs 10: 0.0002

TABLE 9

THE SURVIVAL OF MICE IN DAYS AND THE NUMBER OF FOCI WITH DIAMETERS OF GREATER THAN 1 MILLIMETER AND THE AVERAGE SIZE OF THE METASTATIC FOCI IN THE LUNG AND LIVER OF MICE FOLLOWING INTRAVENOUS INFUSION OF 2, 5, AND 20 X 10^6 LEWIS LUNG CARCINOMA CELLS WITH VIABILITY OF 90% AND WITH 8% AGGREGATED CELLS IN STUDY 1. THE TISSUES WERE EXAMINED AT THE TIME OF DEATH OF THE MICE.

Mouse	Survival Days	Lung			Liver		
		Foci# total	Foci# >1mm	Avg size >1mm	Foci# total	Foci# >1mm	Avg size >1mm
DOSE: 2 X 10^6							
1	19	46		4.14	39		2.73
2	19	19		3.82	6		2.81
3	19	33		4.16	3		2.37
4	19	15		3.32	35		5.12
5	20	9		2.63	78		5.64
6	24	37		4.48	12		4.47
7	25	6		3.41	4		2.56
8	26	20		4.02	6		6.61
Mean	21.4	23.1		3.75	22.9		4.04
SD	3.1	14.1		0.60	26.4		1.64
N	8	8		8	8		8
DOSE: 5 X 10^6							
1	12	5		3.65			
2	15	43		3.37	10		1.63
3	16	31		2.83	46		1.51
4	16	33		4.40	89		3.01
5	17	39		4.02	79		2.76
6	17	15		4.10	11		2.77
7	18	26		3.12	79		5.03
8	21	46		4.92	9		6.96
Mean	16.5	29.8		3.80	46.1		3.38
SD	2.6	14.0		0.69	36.3		1.96
N	8	8		8	7		7
DOSE: 20 X 10^6							
1	12	37		6.50	10		0.94
2	12	30		4.00	11		0.66
3	12	41		5.12	5		0.86
4	12	49		3.35	9		0.56
5	13	43		2.59	19		0.46
6	15	55		5.09	33		1.20
7	10	37		3.40	6		0.48
Mean	13.1	41.7		4.29	13.3		0.74
SD	1.7	8.3		1.35	9.8		0.27
N	7	7		7	7		7

TABLE 10A

THE SURVIVAL OF MICE IN DAYS AND THE NUMBER OF FOCI WITH A DIAMETER OF GREATER THAN 1 MILLIMETER, AND THE AVERAGE SIZE OF THE METASTATIC FOCI FOLLOWING INTRAVENOUS INFUSION OF 2, 5, 10, AND 20 X 10^6 LEWIS LUNG CARCINOMA CELLS WITH IN VITRO VIABILITY OF 90% AND WITH 10% AGGREGATED CELLS IN STUDY 2. THE TISSUES WERE EXAMINED AT THE TIME OF THE DEATH OF THE MICE.

Mouse	Survival Days	Lung			Liver		
		Foci# total	Foci# >1mm	Avg size >1mm	Foci# total	Foci# >1mm	Avg size >1mm
DOSE: 2 X 10^6							
1	17	49		3.66	32		2.10
2	17	30		3.20	29		2.35
3	17	29		3.09	6		2.77
4	18	20		2.54	34		5.27
5	18	21		3.71	12		1.90
6	18	39		3.81	63		5.27
7	18	29		3.77	27		2.97
8	19	26		4.27	6		1.20
9	19	19		3.83	16		3.72
10	20	36		3.02	3		2.55
11	22	29		3.71	82		6.26
Mean	18.5	29.7		3.5	28.2		3.3
SD	1.5	8.9		0.5	24.9		1.6
N	11	11		11	11		11
DOSE: 5 X 10^6							
1	13	79		3.48	76		1.07
2	15	83		3.47	3		0.57
3	15	90		3.62	39		0.96
4	15	46		3.71	79		1.97
5	16	48		4.07	52		2.55
6	16	48		4.13	112		3.30
7	16	33		4.08	53		2.23
8	17	37		4.14	8		2.89
8	19	76		3.00	5		3.68
10	22	15		4.94	0		0.00
Mean	16.4	55.5		3.86	42.7		1.92
SD	2.5	25.0		0.53	38.7		1.23
N	10	10		10	10		10

TABLE 10B

DOSE: 10×10^6					
1	12	93	2.47	40	0.84
2	12	76	2.67	17	0.72
3	12	70	2.35	12	0.81
4	12	84	2.20	65	0.95
5	13	84	2.10	223	1.65
6	13	59	2.03	77	1.00
7	13	114	3.49	52	1.45
8	15	77	3.83	5	1.11
9	15	93	3.61	1	1.42
Mean	13.0	83.3	2.75	54.7	1.11
SD	1.2	15.8	0.70	68.7	0.33
N	9	9	9	9	9
DOSE: 20×10^6					
1	11	87	1.84	103	0.87
2	11	104	1.90	300	1.27
3	11	98	0.90	53	0.75
4	11	101	0.90	25	0.62
5	12	79	1.84	21	0.74
6	12	89	2.03	167	1.10
7	13	103	2.52	154	0.87
8	13	114	2.36	23	1.03
8	14	107	3.12	200	1.35
10	15	117	3.20	21	1.15
Mean	12.3	99.9	2.06	106.7	0.98
SD	1.4	12.0	0.78	96.2	0.24
N	10	10	10	10	10

TABLE 11A

THE SURVIVAL OF MICE IN DAYS AND THE NUMBER OF METASTATIC FOCI GREATER THAN 1 MILLIMETER IN DIAMETER AND THE AVERAGE SIZE OF THE METASTATIC FOCI IN THE LUNG AND LIVER FOLLOWING INTRAVENOUS INFUSION OF 2, 5, 10, AND 20 X 10^6 LEWIS LUNG CARCINOMA CELLS WITH IN VITRO VIABILITY OF 91% AND WITH 5% AGGREGATED CELLS IN STUDY 3. THE TISSUES WERE EXAMINED AT THE TIME OF DEATH OF THE MICE.

Mouse	Survival Days	Lung			Liver		
		Foci# total	Foci# >1mm	Avg size >1mm	Foci# total	Foci# >1mm	Avg size >1mm
DOSE: 2 X 10^6							
1	16	36		3.64	31		2.45
2	16	50		4.14	6		1.53
3	17	28		3.70	1		1.17
4	17	28		3.59	16		1.66
5	18	17		2.59	17		3.70
6	18	33		3.58	2		1.22
7	21	34		4.87	2		1.16
8	21	17		3.31	1		4.59
9	22	34		1.67	8		2.71
10	23	23		1.04	39		4.92
Mean	18.9	30.0		3.2	12.3		2.5
SD	2.6	9.8		1.1	13.4		1.4
N	10	10		10	10		10
DOSE: 5 X 10^6							
1	14	82		3.09	91		2.58
2	15	52		2.67	63		1.87
3	15	61		3.22	28		1.63
4	15	65		2.93	101		1.97
5	16	63		3.42	29		1.86
6	17	40		3.63	52		3.57
7	17	21		3.40	25		3.22
8	17	30		3.10	12		2.85
9	18	46		3.60	50		3.18
Mean	16.0	51.1		3.23	50.1		2.53
SD	1.3	19.0		0.32	30.5		0.72
N	9	9		9	9		9

TABLE 11B

DOSE: 10×10^6					
1	11	85	2.03	200	1.03
2	12	68	2.06	200	1.34
3	12	48	2.27	250	2.05
4	13	60	2.66	30	0.87
5	13	56	2.96	43	1.11
6	14	89	2.75	74	1.41
7	15	68	2.88	105	2.06
8	16	46	2.75	54	1.75
9	16	56	3.36	44	2.31
Mean	13.6	64.0	2.64	111.1	1.55
SD	1.8	15.1	0.44	83.3	0.51
N	9	9	9	9	9
DOSE: 20×10^6					
1	10	93	1.76	300	1.36
2	10	123	1.69	126	0.81
3	10	100	2.49	89	1.01
4	11	129	2.08	90	0.60
5	12	114	2.23	16	0.83
6	12	110	2.67	18	1.01
7	12	76	2.49	134	1.16
8	12	118	2.62	43	0.77
9	13	107	2.95	32	0.85
Mean	11.3	107.8	2.33	94.2	0.93
SD	1.1	16.3	0.42	89.0	0.23
N	9	9	9	9	9

TABLE 12A

THE SURVIVAL OF MICE IN DAYS AND THE NUMBER OF METASTATIC FOCI GREATER THAN 1 MILLIMETER IN DIAMETER AND THE AVERAGE SIZE OF THE METASTATIC FOCI IN THE LUNG, LIVER, SPLEEN, HEART AND KIDNEY FOLLOWING INTRAVENOUS INFUSION OF 1, 5, AND 10 X 10^6 LEWIS LUNG CARCINOMA CELLS IN STUDY 4. THE TISSUES WERE EXAMINED AT THE TIME OF DEATH OF THE MICE. THE TUMOR CELLS WERE ISOLATED USING DIGESTION METHOD 1.

Mouse	Survival Days	Lung			Liver			Spleen	Heart	Kidneys
		Foci# total	Foci# >1mm	Avg size >1mm	Foci# total	Foci# >1mm	Avg size >1mm	Foci # total	Foci # total	Foci # total
DOSE: 1 X 10^6										
1	16	54	11	1.68	7	0	0.00	0	0	0
2	19	38	9	1.80	9	4	1.96	0	1	0
3	21	11	5	2.20	0	0	0.00	0	0	0
4	22	9	1	2.78	4	3	2.45	0	0	0
5	23	7	3	1.60	1	1	3.64	0	0	0
6	24	53	9	2.03	1	1	5.01	0	0	0
7	25	88	6	2.15	0	0	0.00	0	0	0
8	25	75	12	1.79	0	0	0.00	0	2	0
9	25	40	6	1.83	0	3	2.35	0	4	0
10	29	111	17	1.59	0	0	0.00	0	0	0
Mean	22.9	48.6	7.9	1.95	2.2	1.2	1.54	0.0	0.7	0.0
SD	3.6	35.1	4.7	0.36	3.3	1.5	1.82	0.0	1.3	0.0
N	10	10	10	10	10	10	10	10	10	10

TABLE 12B

DOSE: 5 X 10^6

1	13	68	6	1.56	0	0	0.00	0	0	0
2	15	58	6	1.78	0	0	0.00	0	1	0
3	19	43	5	1.74	0	0	0.00	0	0	0
4	19	4	8	1.97	0	0	0.00	0	0	0
5	19	24	7	2.11	0	0	0.00	0	0	0
6	19	54	9	1.88	3	0	0.00	0	0	0
7	19	71	11	1.90	13	4	1.74	0	3	0
8	19	27	5	1.96	7	3	1.98	0	0	0
9	19	14	6	2.33	10	6	1.68	0	0	0
10	19	26	7	1.94	10	3	1.82	0	0	0
Mean	18.0	38.9	7.0	1.92	4.3	1.6	0.72	0.0	0.4	0.0
SD	2.2	23.2	1.9	0.21	5.2	2.2	0.94	0.0	1.0	0.0
N	10	10	10	10	10	10	10	10	10	10

DOSE: 10 X 10^6

1	15	87	17	1.72	3	2	1.31	0	0	0
2	15	57	9	1.64	0	0	0.00	0	1	0
3	15	63	12	1.49	20	3	1.45	0	0	0
4	19	44	7	1.95	41	8	1.82	0	0	0
5	19	51	11	1.91	16	4	1.92	0	0	0
6	19	28	6	1.96	8	3	2.07	0	0	0
7	20	93	12	1.43	98	12	1.70	0	0	0
8	20	71	9	1.80	71	9	1.64	0	0	0
9	20	56	12	1.80	14	2	3.00	0	0	0
10	20	59	11	2.12	29	8	2.02	0	0	0
Mean	18.2	60.9	10.6	1.78	30.0	5.1	1.69	0.0	0.1	0.0
SD	2.3	19.2	3.1	0.22	31.8	3.9	0.75	0.0	0.4	0.0
N	10	10	10	10	10	10	10	8	8	8

TABLE 13

CORRELATIONS BETWEEN THE NUMBER OF METASTATIC FOCI IN THE LUNG, 125IUDR RADIOACTIVITY IN THE LUNG, AND WEIGHT OF THE LUNGS AND LIVER IN MICE SACRIFICED ON THE DAY FOLLOWING INFUSION OF 1, 2, 5, 10, AND 20 x 10^6 LEWIS LUNG CARCINOMA CELLS

Correlation Between Number of Tumor Cells Infused and:	Correlation Coefficient	p Value
Number of tumor foci in the lung	0.914	0.001
125I radioactivity in the lung	0.828	0.001
Weight of lung and liver	0.884	0.001

TABLE 14A

THE 125-IUDR RADIOACTIVITY (CPM) AND 125-IUDR RADIOACTIVITY PER GRAM OF WEIGHT IN THE LUNG, LIVER, AND SPLEEN IN MICE SACRIFICED ON DAYS 9 OR 12 FOLLOWING INTRAVENOUS INFUSION OF 0, 2, 5, 10, AND 20 X 106 LEWIS LUNG CARCINOMA CELLS

TUMOR CELLS INFUSED ($X 10^6$):		CPM/ORGAN					CPM/GRAM ORGAN WEIGHT				
		0	2	5	10	20	0	2	5	10	20
LUNG											
STUDY 1	MEAN	93	261	546	790	3066	410	1340	2480	3352	8537
	SD	34	63	319	285	1302	171	339	1324	981	2359
	N	9	11	9	9	8	9	11	9	9	8
STUDY 2	MEAN	105	154	244	494	1088	508	612	875	1680	3153
	SD	31	65	114	112	1402	158	176	288	762	2916
	N	10	10	10	9	10	10	10	10	9	10
STUDY 3	MEAN	144	215	281	324	823	719	913	1001	1486	3558
	SD	36	61	36	128	433	168	273	162	539	1772
	N	9	10	10	8	10	9	10	10	7	10
LIVER											
STUDY 1	MEAN	1009	1267	936	1247	2346	588	1030	808	1037	2768
	SD	338	326	319	299	1976	175	237	288	216	2676
	N	9	11	9	9	8	9	11	9	9	8
STUDY 2	MEAN	1000	1127	1122	1212	2451	567	614	551	652	1481
	SD	254	277	362	468	1763	160	189	169	264	1232
	N	10	10	10	9	10	10	10	10	9	10
STUDY 3	MEAN	1518	1224	1111	1656	1245	859	665	622	892	672
	SD	379	249	178	488	258	246	121	131	254	129
	N	9	10	10	8	10	9	10	10	8	10

TABLE 14B

SPLEEN											
STUDY 1	MEAN	776	848	863	1080	2645	5090	9176	10194	12496	27215
	SD	870	564	403	522	1936	4827	3345	3286	4667	14019
	N	9	11	9	9	8	9	11	9	9	8
STUDY 2	MEAN	767	556	925	1013	1006	5824	4650	7819	9463	9514
	SD	532	620	491	660	759	3027	3827	2331	4785	7299
	N	10	10	10	9	10	10	10	10	9	10
STUDY 3	MEAN	1177	812	1856	1755	1963	10168	7786	13760	14304	13126
	SD	539	482	1002	784	871	4413	4658	6964	6387	5942
	N	9	10	10	8	9	9	10	10	8	9

TABLE 15

THE NUMBER OF METASTATIC FOCI GREATER THAN 1 MILLIMETER IN DIAMETER IN THE LUNGS AND LIVER OF MICE SACRIFICED ON DAY 9 OR 12 FOLLOWING INFUSION OF 2, 5, 10, AND 20 X 106 LEWIS LUNG CARCINOMA CELLS

INFUSED	TUMOR CELLS (MILLIONS)		NUMBER OF TUMOR FOCI 2	5	10	20
LUNGS	STUDY 1*	MEAN	6	30	37	77
		SD	4	7	10	15
		N	11	9	9	8
	STUDY 2**	MEAN	5	15	41	69
		SD	6	16	16	15
		N	10	10	9	10
	STUDY 3**	MEAN	7	26	54	110
		SD	4	12	23	16
		N	10	10	8	10
LIVER	STUDY 1*	MEAN	0	0	0	2
		SD	0	0	0	3
		N	11	9	9	8
	STUDY 2**	MEAN	0	2	4	53
		SD	0	2	4	59
		N	10	10	9	10
	STUDY 3**	MEAN	1	3	6	29
		SD	1	2	5	13
		N	10	10	8	10

*STUDY 1: SACRIFICED DAY 12
**STUDIES 2 AND 3: SACRIFICED DAY 9

TABLE 16A

THE WEIGHT, NUMBER OF METASTATIC FOCI, MEAN DIAMETER OF METASTATIC FOCI, SMALLEST DIAMETER OF METASTATIC FOCI, THE 125-IUDR RADIOACTIVITY (CPM), AND THE 125-IUDR RECOVERY PER GRAM OF TISSUE IN THE LUNG, LIVER, AND SPLEEN OF MICE SACRIFICED ON DAY 12 FOLLOWING THE INFUSION OF 2, 5, 10 and 20 X 10^6 LEWIS LUNG CARCINOMA CELLS IN STUDY 1

	LUNG						LIVER						SPLEEN		
DOSE	organ weight g	foci #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE: 2 X 10^6															
	0.140	4	1.00	0.00	301	2154	1.155	0	0.00	0.00	1231	1065	0.074	863	11666
	0.198	15	0.97	0.49	258	1305	1.310	0	0.00	0.00	840	641	0.090	630	7001
	0.188	0	0.00	0.00	170	903	1.325	0	0.00	0.00	1202	907	0.070	699	9985
	0.170	3	0.84	0.00	191	1126	1.170	0	0.00	0.00	966	826	0.065	537	8264
	0.131	4	0.81	0.00	185	1414	1.010	0	0.00	0.00	898	889	0.060	540	8994
	0.192	14	0.93	0.52	321	1673	1.130	0	0.00	0.00	1462	1294	0.150	2279	15194
	0.220	8	0.71	0.32	286	1301	1.190	0	0.00	0.00	1216	1022	0.068	396	5827
	0.190	5	0.63	0.00	202	1062	1.270	0	0.00	0.00	1055	831	0.105	1480	14093
	0.232	7	1.11	0.39	328	1414	1.260	0	0.00	0.00	1779	1412	0.110	826	7512
	0.250	3	0.95	0.00	282	1130	1.320	0	0.00	0.00	1557	1180	0.090	730	8115
	0.270	4	1.04	0.00	341	1263	1.370	0	0.00	0.00	1731	1264	0.080	343	4284
Mean	0.198	6	0.82	0.16	261	1340	1.228	0	0.00	0.00	1267	1030	0.087	848	9176
SD	0.043	5	0.31	0.22	63	339	0.107	0	0.00	0.00	326	237	0.026	564	3345
N	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
DOSE: 5 X 10^6															
	0.230	31	1.91	0.45	768	3339	1.185	0	0.00	0.00	1074	906	0.096	1548	16126
	0.219	42	1.70	0.54	1088	4967	0.970	0	0.00	0.00	489	504	0.070	753	10750
	0.265	38	2.29	0.69	977	3687	1.423	0	0.00	0.00	918	645	0.120	1402	11680
	0.170	30	1.39	0.68	485	2856	1.233	0	0.00	0.00	841	682	0.063	327	5196
	0.208	29	1.52	0.41	306	1470	1.150	0	0.00	0.00	886	770	0.065	565	8699
	0.210	31	1.17	0.34	279	1327	1.245	0	0.00	0.00	1054	847	0.081	778	9609
	0.215	32	1.70	0.35	258	1199	1.085	0	0.00	0.00	684	631	0.074	576	7783
	0.210	21	1.47	0.56	460	2189	1.090	0	0.00	0.00	1640	1505	0.079	1076	13614
	0.229	20	1.74	0.39	295	1290	1.080	0	0.00	0.00	841	779	0.089	738	8289
Mean	0.217	30	1.65	0.49	546	2480	1.162	0	0.00	0.00	936	808	0.082	863	10194
SD	0.025	7	0.32	0.13	319	1324	0.130	0	0.00	0.00	319	288	0.018	403	3286
N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

TABLE 16B

	LUNG						LIVER						SPLEEN		
	organ weight g	foci #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE:	10×10^6														
	0.172	23	1.12	0.30	413	2400	1.167	0	0.00	0.00	773	662	0.060	486	8101
	0.168	27	1.45	0.41	557	3315	1.157	0	0.00	0.00	1556	1345	0.082	716	8734
	0.199	32	1.62	0.34	828	4162	1.301	0	0.00	0.00	1179	906	0.078	753	9648
	0.208	23	1.51	0.60	1035	4976	1.260	0	0.00	0.00	1380	1095	0.085	1459	17163
	0.210	38	1.73	0.32	778	3704	1.140	0	0.00	0.00	1510	1325	0.085	1736	20428
	0.260	49	1.86	0.57	474	1822	1.490	0	0.00	0.00	1602	1075	0.110	1795	16317
	0.333	48	1.61	0.36	1320	3963	1.195	0	0.00	0.00	1285	1076	0.085	577	6783
	0.355	43	2.64	0.43	902	2541	1.100	0	0.00	0.00	1076	978	0.110	1455	13229
	0.244	47	2.82	0.45	802	3286	0.990	0	0.00	0.00	861	869	0.062	748	12058
Mean	0.239	37	1.82	0.42	790	3352	1.200	0	0.00	0.00	1247	1037	0.084	1080	12496
SD	0.067	11	0.56	0.11	285	981	0.141	0	0.00	0.00	299	216	0.018	522	4667
N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
DOSE:	20×10^6														
	0.215	58	2.63	0.36	997	4639	0.960	0	0.00	0.00	439	457	0.095	1138	11982
	0.342	92	2.55	0.54	2931	8569	0.880	0	0.00	0.00	614	698	0.121	5023	41515
	0.385	77	3.67	0.91	4113	10684	0.850	0	0.00	0.00	967	1138	0.082	3336	40685
	0.355	54	3.96	0.47	3193	8994	0.822	0	0.00	0.00	1377	1675	0.125	5926	47408
	0.380	95	2.91	0.44	3735	9829	0.812	0	0.00	0.00	3610	4446	0.089	2312	25974
	0.530	68	3.08	0.39	4954	9348	0.720	10	2.65	0.99	6003	8337	0.039	786	20163
	0.283	76	2.02	0.51	3104	10969	1.020	4	0.43	0.00	3958	3880	0.086	1319	15340
	0.285	97	2.19	0.41	1501	5268	1.190	0	0.00	0.00	1799	1512	0.09	1319	14658
Mean	0.347	77	2.88	0.50	3066	8537	0.907	2	0.39	0.12	2346	2768	0.091	2645	27215
SD	0.094	17	0.68	0.17	1302	2359	0.147	4	0.93	0.35	1976	2676	0.026	1936	14019
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

TABLE 17A

THE WEIGHT, NUMBER OF METASTATIC FOCI, MEAN DIAMETER OF METASTATIC FOCI, SMALLEST DIAMETER OF METASTATIC FOCI, THE 125-IUDR RADIOACTIVITY (CPM), AND THE 125-IUDR RECOVERY PER GRAM OF TISSUE IN THE LUNG, LIVER, AND SPLEEN OF MICE SACRIFICED ON DAY 9 FOLLOWING THE INFUSION OF 2, 5, 10, and 20 X 10^6 LEWIS LUNG CARCINOMA CELLS IN STUDY 2.

STUDY 2.

	LUNG							LIVER							SPLEEN		
	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE: 2 X 10^6																	
	0.182	5	0	0.37	0.00	70	386	1.680	0	0	0.00	0.00	1094	651	0.080	23	293
	0.220	11	0	0.49	0.29	149	679	1.945	0	0	0.00	0.00	1379	709	0.100	550	5496
	0.245	14	0	0.57	0.34	122	496	2.155	0	0	0.00	0.00	631	293	0.130	574	4417
	0.250	0	0	0.00	0.00	143	571	1.985	0	0	0.00	0.00	1106	557	0.100	744	7436
	0.230	0	0	0.00	0.00	114	494	1.835	0	0	0.00	0.00	1123	612	0.100	22	223
	0.240	9	0	0.37	0.30	164	683	1.800	0	0	0.00	0.00	994	552	0.080	474	5923
	0.230	11	0	0.39	0.29	216	940	1.690	0	0	0.00	0.00	1433	848	0.110	546	4966
	0.330	0	0	0.00	0.00	258	780	1.680	0	0	0.00	0.00	1591	947	0.090	420	4670
	0.185	5	0	0.38	0.00	74	398	1.800	0	0	0.00	0.00	962	535	0.120	46	381
	0.340	0	0	0.00	0.00	235	692	2.185	0	0	0.00	0.00	960	439	0.170	2158	12697
Mean	0.245	6	0	0.26	0.12	154	612	1.876	0	0	0.00	0.00	1127	614	0.108	556	4650
SD	0.053	5	0	0.23	0.16	65	176	0.187	0	0	0.00	0.00	277	189	0.027	620	3827
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
DOSE: 5 X 10^6																	
	0.210	8	0	0.42	0.32	217	1035	1.860	5	0	0.36	0.00	1339	720	0.090	601	6677
	0.200	8	1	0.80	0.34	159	797	1.960	0	0	0.00	0.00	1544	788	0.110	719	6537
	0.193	19	0	0.54	0.29	134	693	1.690	0	0	0.00	0.00	768	455	0.095	693	7300
	0.280	3	0	0.33	0.00	221	788	1.840	0	0	0.00	0.00	696	378	0.075	504	6719
	0.330	10	0	0.59	0.37	265	804	2.120	5	0	0.32	0.00	1038	490	0.160	1656	10348
	0.290	20	0	0.60	0.38	439	1515	2.130	0	0	0.00	0.00	1188	558	0.145	1700	11726
	0.410	55	0	0.66	0.36	439	1071	2.490	4	0	0.36	0.00	1046	420	0.150	1420	9469
	0.290	19	0	0.72	0.47	265	915	2.190	3	0	0.44	0.00	1840	840	0.100	909	9086
	0.340	0	0	0.00	0.00	151	443	2.020	0	0	0.00	0.00	875	433	0.110	730	6639
	0.210	10	0	0.36	0.29	145	690	2.050	0	0	0.00	0.00	883	431	0.085	313	3686
Mean	0.275	15	0	0.50	0.28	244	875	2.035	2	0	0.15	0.00	1122	551	0.112	925	7819
SD	0.072	16	0	0.23	0.16	114	288	0.221	2	0	0.19	0.00	362	169	0.030	491	2331
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE 17B

	LUNG							LIVER							SPLEEN		
	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE: 10 X 10^6																	
	0.210	37	0	0.63	0.29	463	2203	1.465	4	0	0.37	0.00	1313	896	0.080	510	6369
	0.246	19	[illegible]	0.82	0.33	433	1758	1.690	10	0	0.43	0.28	1019	603	0.070	415	5925
	0.310	44	0	0.65	0.42	527	1701	1.870	4	0	0.46	0.00	1356	725	0.105	1074	10225
	0.250	39	1	0.90	0.42	482	1927	1.680	7	0	0.39	0.32	1602	954	0.090	776	8622
	0.230	31	0	0.58	0.37	608	2642	2.130	10	0	0.71	0.28	1597	750	0.118	1919	16261
	0.310	39	0	0.57	0.39	596	1924	2.420	0	0	0.00	0.00	1521	628	0.136	2106	15486
	0.310	29	3	0.95	0.38	253	816	1.720	1	0	0.32	0.00	1201	698	0.095	816	8591
	0.270	75	7	1.49	0.34	608	2250	2.220	0	0	0.00	0.00	1126	507	0.130	1416	10892
	0.300	53	0	0.79	0.41	473	1576	1.820	0	0	0.00	0.00	1386	761	0.090	1104	12264
Mean	0.271	41	1	0.82	0.37	494	1680	1.702	4	0	0.27	0.09	1212	652	0.091	1013	9463
SD	0.039	16	2	0.29	0.04	112	762	0.664	4	0	0.25	0.14	468	264	0.038	660	4785
N	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
DOSE: 20 X 10^6																	
	0.245	49	0	0.45	0.28	525	2143	1.410	8	0	0.36	0.31	1627	1154	0.080	464	5797
	0.235	55	0	0.57	0.32	896	3814	1.810	83	0	0.91	0.29	3754	2074	0.095	1102	11595
	0.250	70	2	1.08	0.39	619	2475	1.910	79	0	0.60	0.41	2379	1246	0.150	1337	8912
	0.290	67	2	1.16	0.40	114	392	1.710	65	0	0.57	0.29	981	574	0.160	37	230
	0.312	76	11	1.63	0.41	693	2223	1.875	20	0	0.54	0.28	1269	677	0.145	640	4413
	0.460	96	16	1.46	0.44	4969	10802	1.445	200	0	0.76	0.32	6816	4717	0.112	2128	19003
	0.320	89	10	1.43	0.36	136	425	1.730	5	0	0.48	0.00	823	476	0.090	33	372
	0.320	55	1	0.91	0.34	891	2784	1.920	31	0	0.60	0.37	2590	1349	0.095	1705	17944
	0.300	63	4	2.10	0.41	998	3326	1.610	20	0	0.61	0.29	2371	1473	0.100	1949	19488
	0.330	74	11	1.35	0.40	1039	3149	1.770	17	0	0.57	0.38	1901	1074	0.090	664	7383
Mean	0.306	69	6	1.21	0.38	1088	3153	1.719	53	0	0.60	0.29	2451	1481	0.112	1006	9514
SD	0.064	15	6	0.49	0.05	1402	2916	0.181	59	0	0.15	0.11	1763	1232	0.029	759	7299
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE 18A

THE WEIGHT, NUMBER OF METASTATIC FOCI, MEAN DIAMETER OF METASTATIC FOCI, SMALLEST DIAMETER OF METASTATIC FOCI, THE 125-IUDR RADIOACTIVITY (CPM), AND THE 125-IUDR PER GRAM OF TISSUE IN THE LUNG, LIVER, AND SPLEEN OF MICE SACRIFICED ON DAY 9 FOLLOWING THE INFUSION OF 2, 5, 10 AND 20 X 10^6 LEWIS LUNG CARCINOMA CELLS IN STUDY 3
STUDY 3.

	LUNG							LIVER							SPLEEN		
	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE: 2 X 10^6																	
	0.220	12	0	0.68	0.47	193	876	1.530	0	0	0.00	0.00	1156	755	0.095	1264	13300
	0.220	9	0	0.74	0.40	247	1121	1.750	1	0	0.34	0.00	1512	864	0.115	1690	14694
	0.250	11	4	1.18	0.41	207	826	2.000	3	0	0.55	0.00	1319	660	0.120	533	4439
	0.220	7	0	0.84	0.37	321	1458	1.900	3	0	0.39	0.00	1375	724	0.070	611	8733
	0.270	0	0	0.00	0.00	246	911	1.740	1	0	0.39	0.00	866	498	0.070	676	9658
	0.170	10	2	1.01	0.27	203	1193	1.690	0	0	0.00	0.00	1201	711	0.130	806	6203
	0.260	5	2	0.93	0.00	172	661	2.000	1	0	0.42	0.00	1233	616	0.101	354	3503
	0.240	4	0	0.63	0.00	130	543	2.110	0	0	0.00	0.00	1086	515	0.180	686	3812
	0.190	9	3	0.98	0.44	140	738	1.600	2	0	0.43	0.00	864	540	0.110	1360	12362
	0.360	6	2	1.03	0.47	288	799	2.130	0	0	0.00	0.00	1627	764	0.120	139	1156
Mean	0.240	7	1	0.80	0.28	215	913	1.845	1	0	0.25	0.00	1224	665	0.111	812	7786
SD	0.052	4	1	0.33	0.20	61	273	0.212	1	0	0.22	0.00	249	121	0.032	482	4658
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
DOSE: 5 X 10^6																	
	0.340	22	4	1.09	0.32	280	823	1.800	4	0	0.40	0.00	1261	701	0.130	36	279
	0.290	7	0	0.69	0.41	304	1047	1.495	1	0	0.46	0.00	1242	831	0.095	1920	20214
	0.340	24	4	1.26	0.48	301	887	1.720	0	0	0.00	0.00	919	534	0.110	2410	21908
	0.240	23	11	1.14	0.55	300	1250	1.940	1	0	0.56	0.00	966	498	0.120	2271	18926
	0.270	32	7	0.97	0.36	250	925	1.990	1	0	0.62	0.00	1287	647	0.110	727	6609
	0.210	52	15	1.46	0.39	224	1068	2.070	7	0	0.47	0.27	810	391	0.355	3473	9783
	0.300	32	16	1.20	0.49	301	1002	1.810	4	0	0.52	0.00	1018	562	0.130	1767	13592
	0.270	33	12	1.37	0.32	345	1279	1.860	2	0	0.65	0.00	1258	676	0.110	1486	13505
	0.280	22	4	1.46	0.39	248	887	1.730	2	0	0.49	0.00	1057	611	0.140	2914	20814
	0.310	13	3	1.08	0.35	261	841	1.685	4	0	0.40	0.00	1290	765	0.130	1556	11973
Mean	0.285	26	8	1.17	0.41	281	1001	1.810	3	0	0.46	0.03	1111	622	0.143	1856	13760
SD	0.041	12	6	0.24	0.08	36	162	0.166	2	0	0.18	0.09	178	131	0.076	1002	6964
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE 18B

	LUNG							LIVER							SPLEEN		
	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight g	foci #	foci >1mm #	mean diam. mm	smallest diam. mm	125I CPM	125I CPM/g	organ weight	125I CPM	125I CPM/g
DOSE: 10 X 10^6																	
	0.210	99	32	1.32	0.42	517	2463	1.770	5	0	0.40	0.00	1866	1054	0.110	1757	15972
		35		1.46	0.39	203		2.070	5	0	0.41	0.00	1573	760	0.120	868	7234
	0.210	42	11	1.22	0.28	254	1208	1.920	2	0	0.48	0.00	1907	993	0.142	2064	14539
	0.200	39	3	1.17	0.28	243	1214	1.610	16	0	0.49	0.33	1185	736	0.105	1313	12503
	0.245	56	16	1.53	0.47	519	2118	1.920	4	0	0.78	0.00	2419	1260	0.140	2146	15330
	0.260	40	4	1.47	0.40	342	1313	1.810	1	0	0.39	0.00	2061	1139	0.095	2175	22900
	0.230	43	12	1.29	0.50	294	1277	1.905	7	0	0.47	0.40	1080	567	0.140	3057	21833
	0.270	76	26	1.87	0.33	219	811	1.840	4	0	0.65	0.00	1152	626	0.150	661	4410
Mean	0	54	14	1.42	0.38	324	1486	1.856	6	0	0.51	0.09	1656	892	0.125	1755	14340
SD	0.03	23	10	0.22	0.08	128	539	0.135	5	0	0.14	0.17	488	254	0.020	784	6387
N	7	8	8	8	8	8	7	8	8	8	8	8	8	8	8	8	8
DOSE: 20 X 10^6																	
	0.190	111	29	1.43	0.29	499	2625	1.680	22	0	0.56	0.25	1039	619	0.133	2528	19006
	0.240	125	5	1.39	0.31	846	3527	1.835	46	0	0.58	0.28	1504	820	0.140	1974	14102
	0.230	86	5	1.70	0.43	871	3787	1.780	7	0	0.60	0.41	1111	624	0.120	1887	15726
	0.220	96	5	1.45	0.37	280	1272	1.870	32	0	0.59	0.30	1159	620	0.190	952	5011
	0.210	94	5	1.97	1.28	531	2529	1.873	29	0	0.56	0.31	970	518	0.150	2430	16199
	0.260	113	27	1.85	0.34	1001	3852	1.830	22	0	0.49	0.32	1405	768	0.180	2285	12694
	0.235	128	27	1.82	0.28	816	3471	1.922	48	0	0.54	0.31	1801	937	0.172	3406	19801
	0.265	104	40	1.76	0.30	1550	5847	1.895	35	0	0.55	0.35	1159	612	0.130	1745	13426
	0.210	137	24	1.70	0.44	1476	7030	1.820	30	0	0.63	0.33	1031	566	0.210	456	2170
	0.220	106	32	1.66	0.26	361	1640	1.990	16	0	0.53	0.40	1270	638			
Mean	0.228	110	20	1.67	0.43	823	3558	1.850	29	0	0.56	0.33	1245	672	0.158	1963	13126
SD	0.023	16	13	0.19	0.30	433	1772	0.084	13	0	0.04	0.05	258	129	0.031	871	5942
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9

EFFECTS OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, ON PRIMARY AND METASTATIC GROWTH OF THE LEWIS LUNG CARCINOMA CELLS

LISTING OF FIGURES

Mice transfused with syngeneic fresh red blood cells, liquid-preserved red blood cells or saline, followed by infusion of tumor cells

1. Survival time of the mice:
1 million tumor cells infused, digestion method 1

2. Survival time of the mice:
5 million tumor cells infused, digestion method 2

3. Survival time of the mice:
2 million tumor cells infused, digestion method 2

4. Survival time of the mice:
2 million tumor cells infused, digestion method 2

5. Survival time of the mice:
2 million tumor cells infused, digestion method 2

6. Survival time of the mice:
1 million tumor cells infused, digestion method 2

7. Survival time of the mice:
1 million tumor cells infused, digestion method 2

Non-transfused mice intravenously infused with increasing numbers of tumor cells isolated by Digestion Method 2.

8. Number of lung foci at the time of death and tumor cell dose infused

9. Size of lung foci at the time of death and tumor cell dose infused

10. Number of liver foci at the time of death and tumor cell dose infused

11. 125IUDR uptake in the lungs at the time of sacrifice and tumor cell dose infused

12. 125IUDR uptake in the liver at the time of sacrifice tumor cell dose infused

13. 125IUDR uptake in the spleen at the time of sacrifice and tumor cell dose infused

FIGURE 1

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 1 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 1

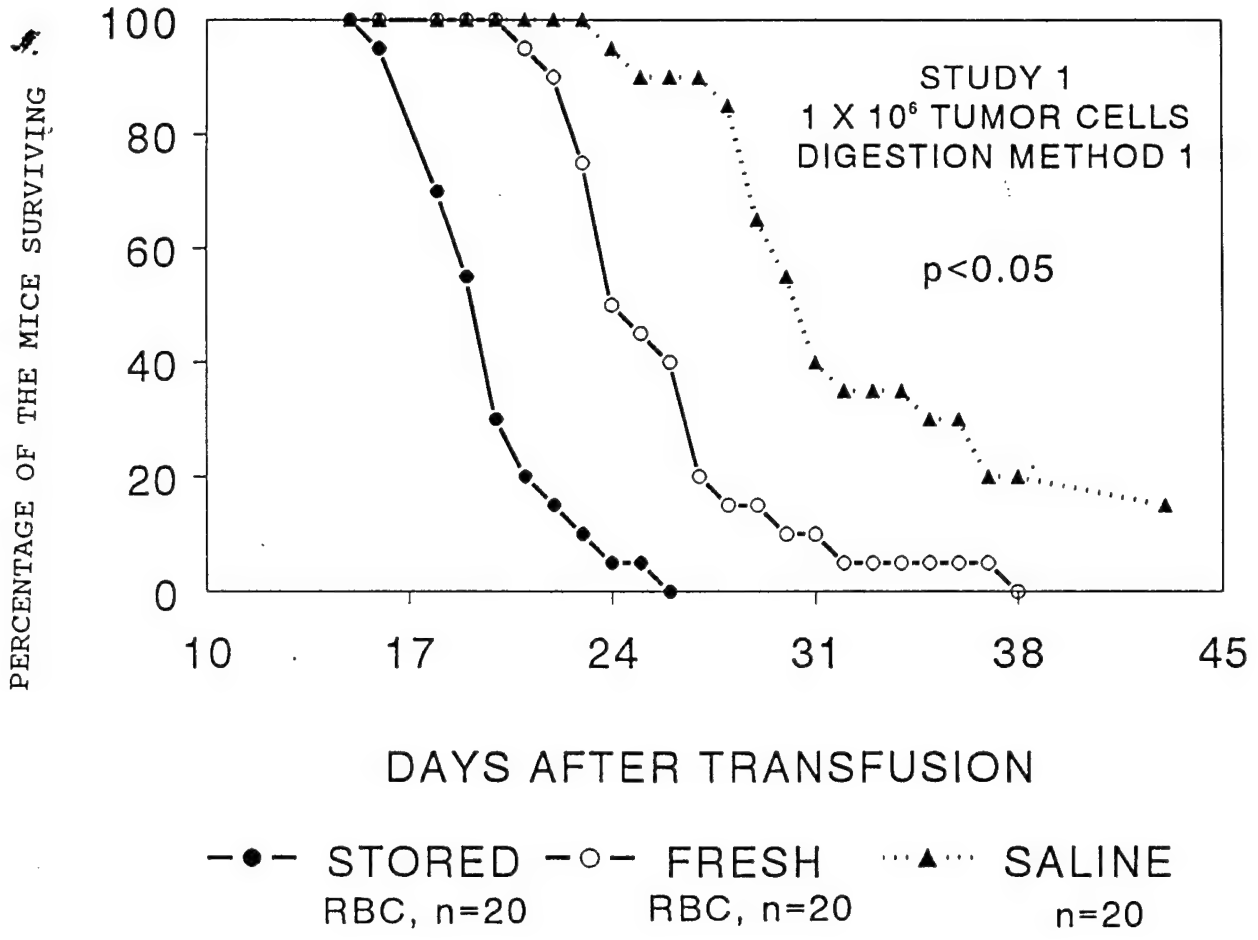


FIGURE 2

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 5 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2

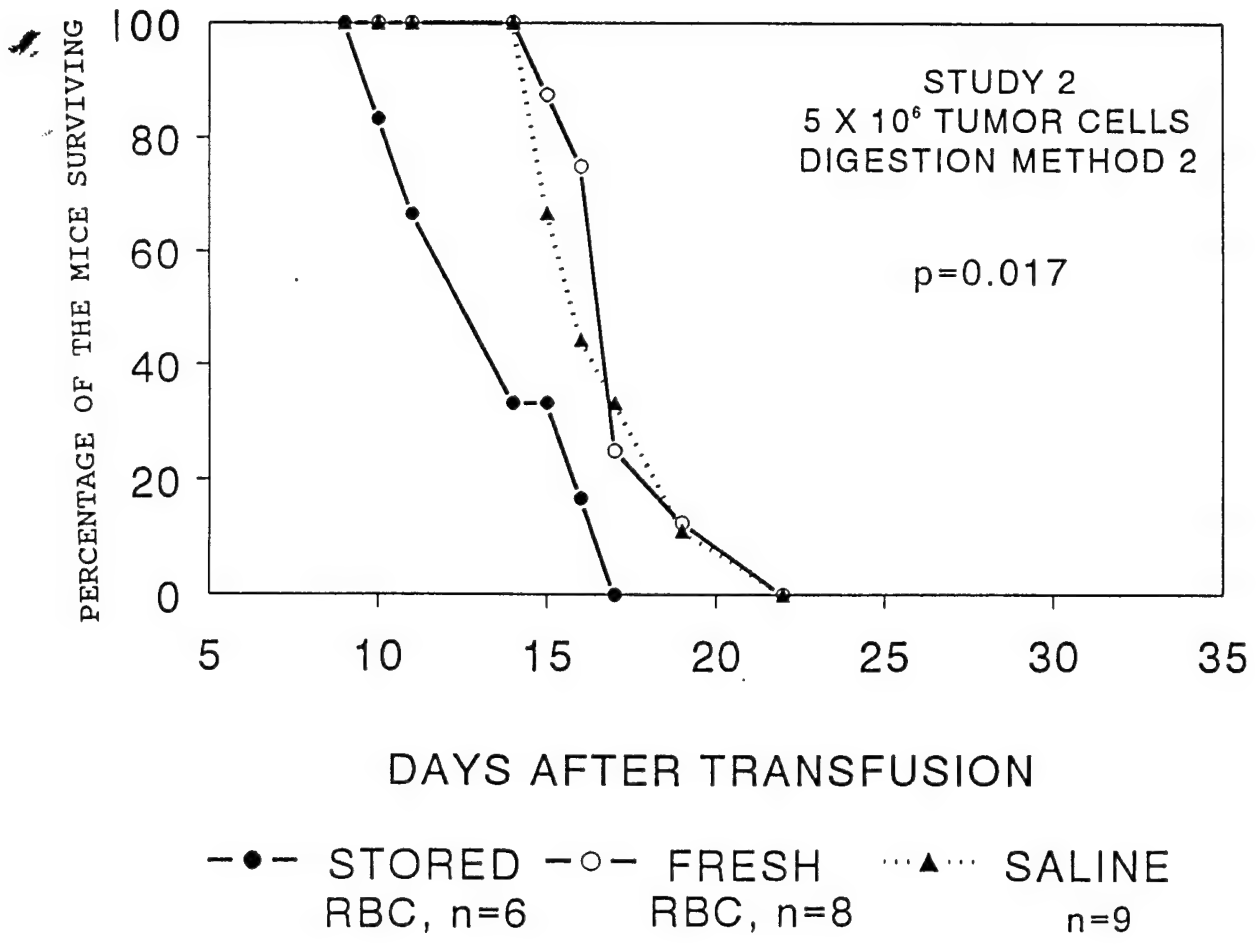


FIGURE 3

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 2 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2

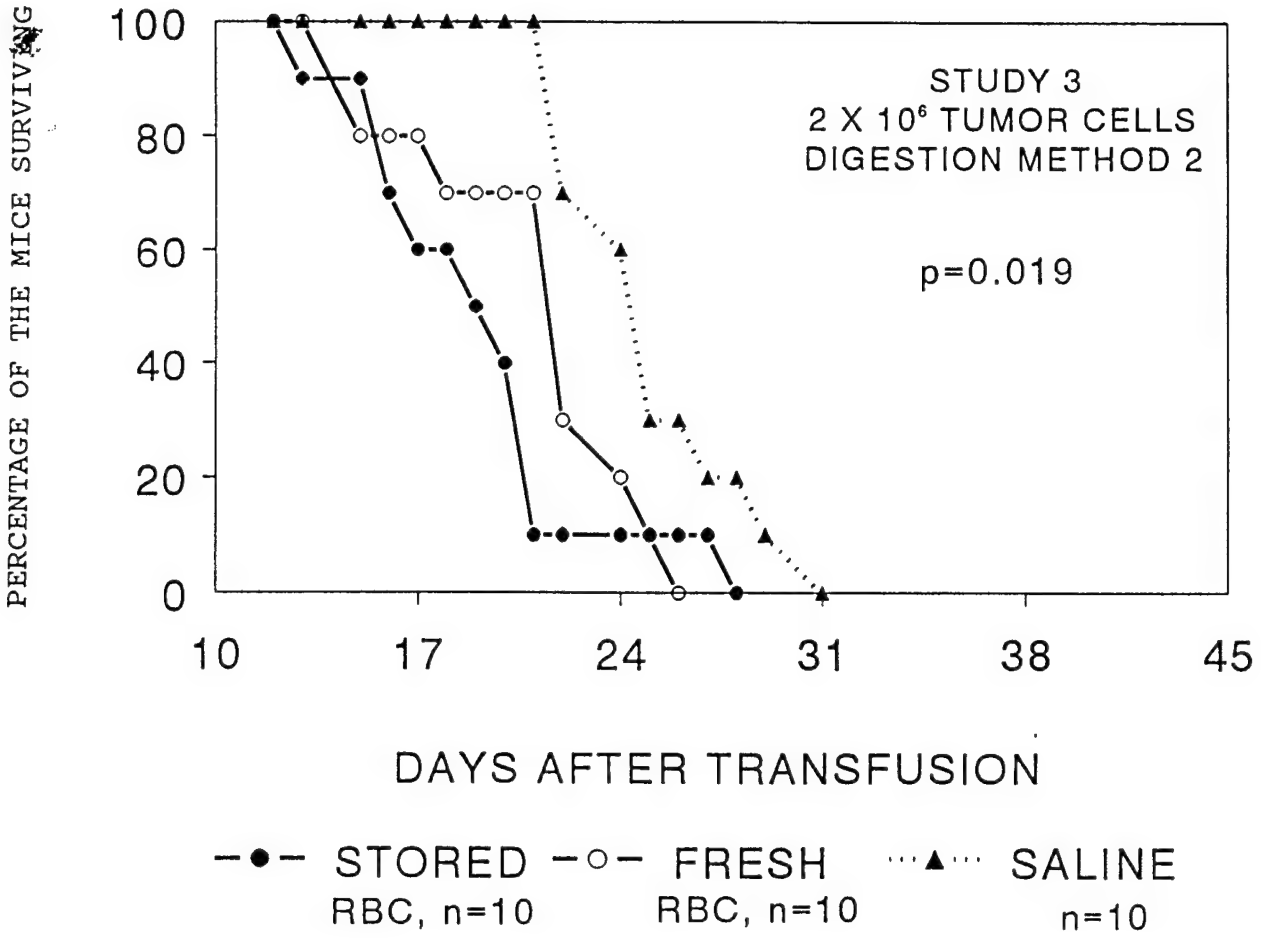
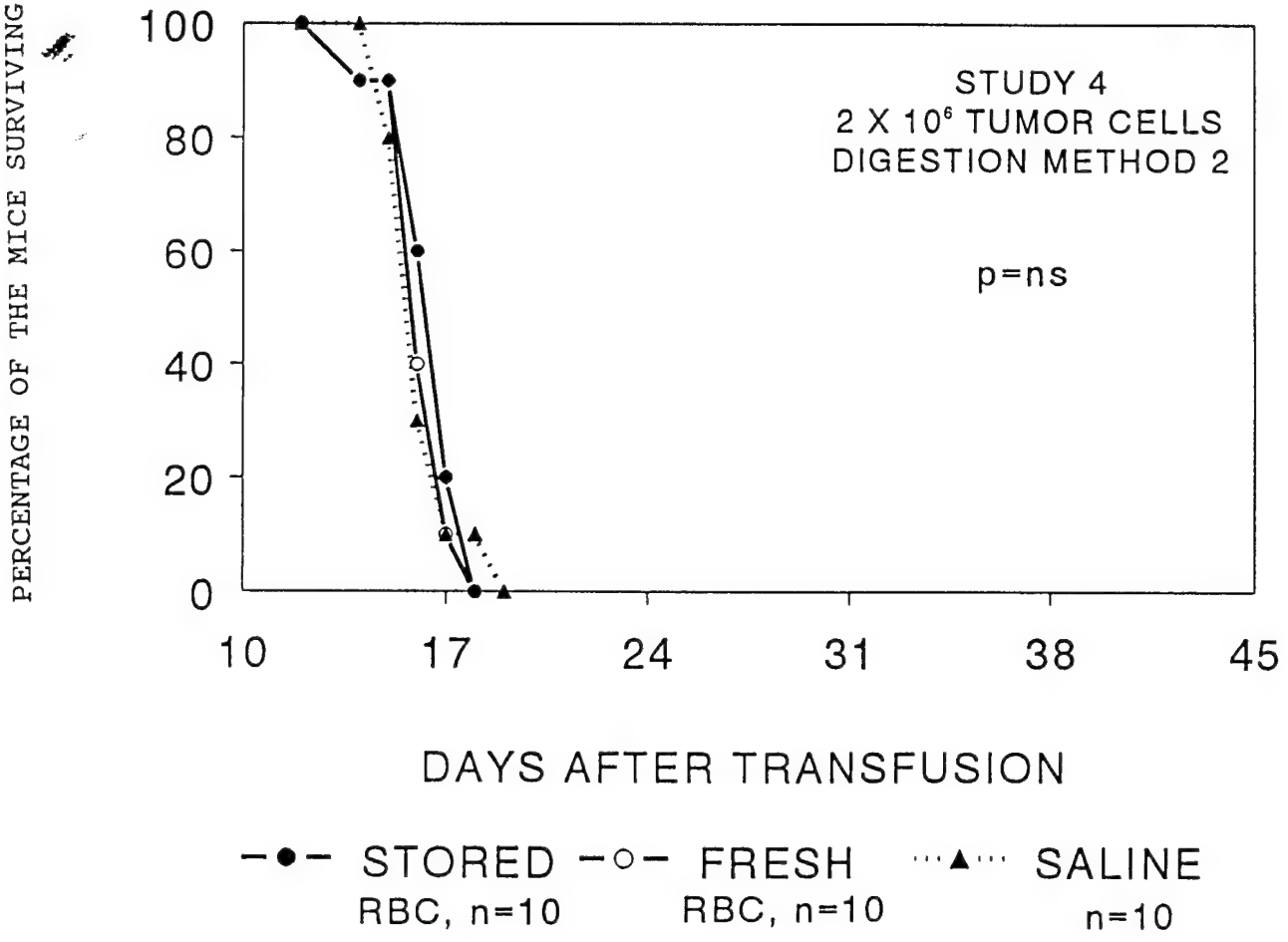


FIGURE 4

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 2 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2



SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 2 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2

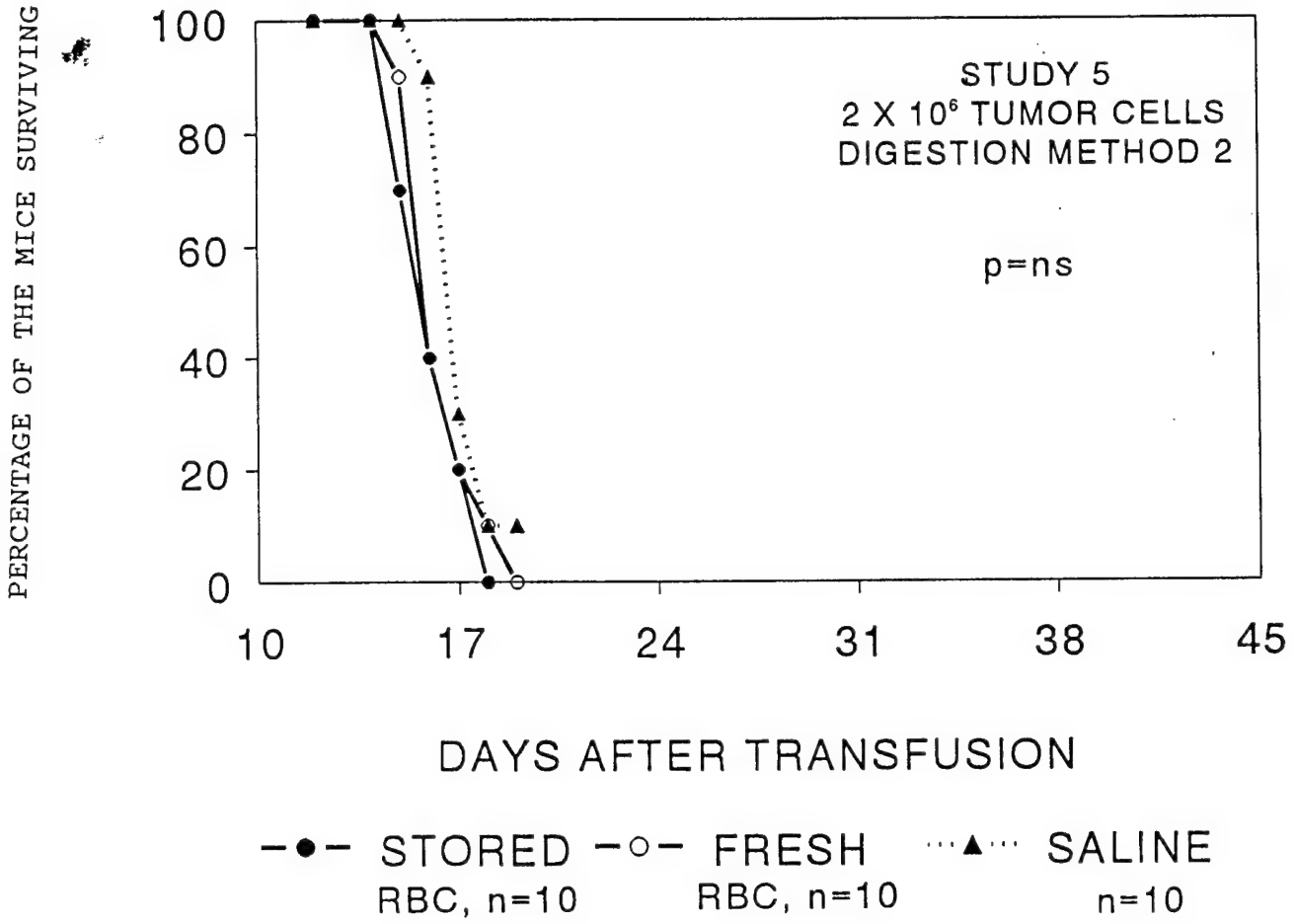


FIGURE 6

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 1 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2

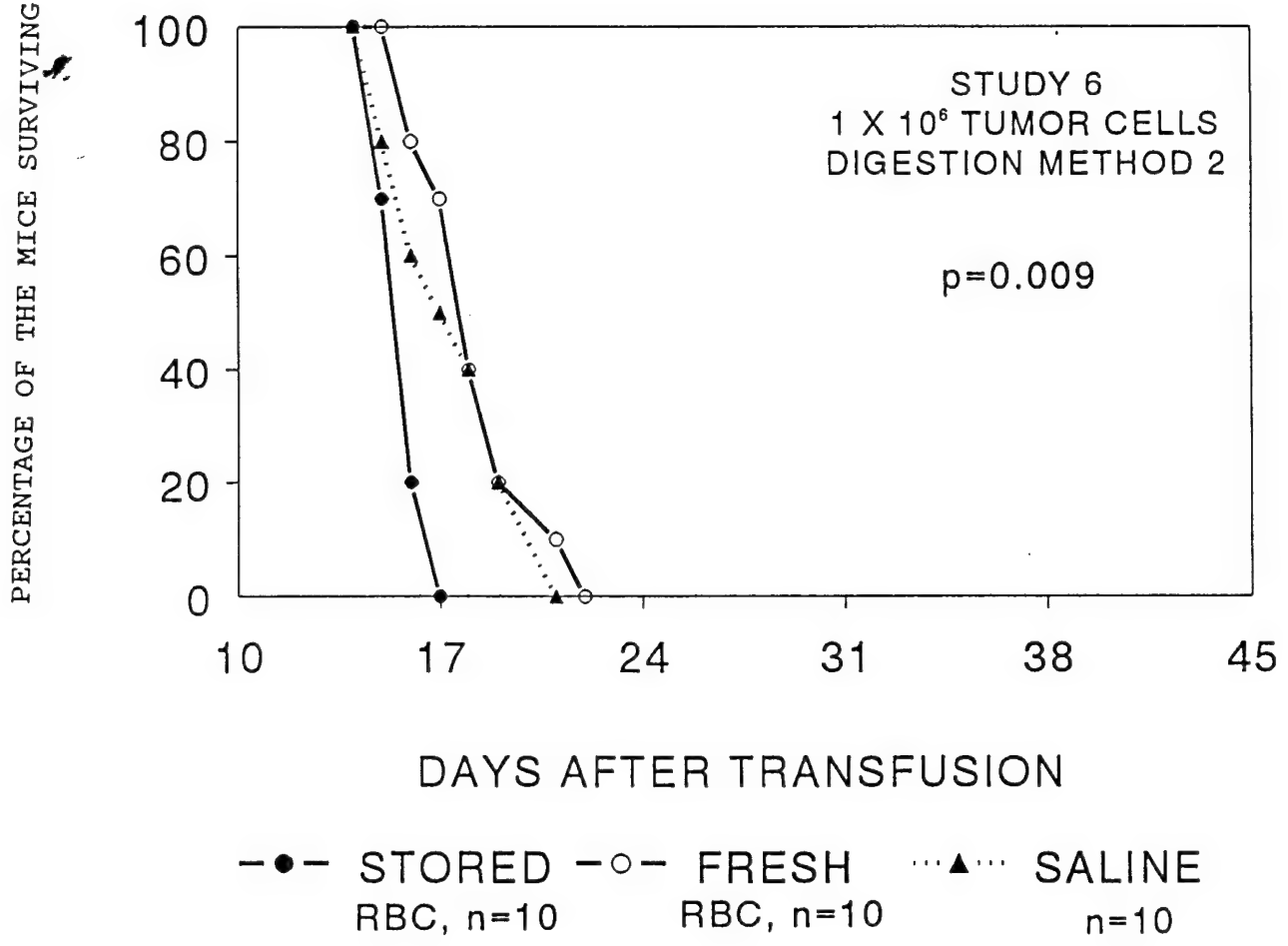


FIGURE 7

SURVIVAL OF MICE FOLLOWING TRANSFUSION OF SYNGENEIC FRESH RED BLOOD CELLS, LIQUID-PRESERVED RED BLOOD CELLS, OR SALINE, AND THEN 4 HOURS LATER WITH THE INTRAVENOUS INFUSION OF 1 X 10^6 TUMOR CELLS PROCESSED BY DIGESTION METHOD 2

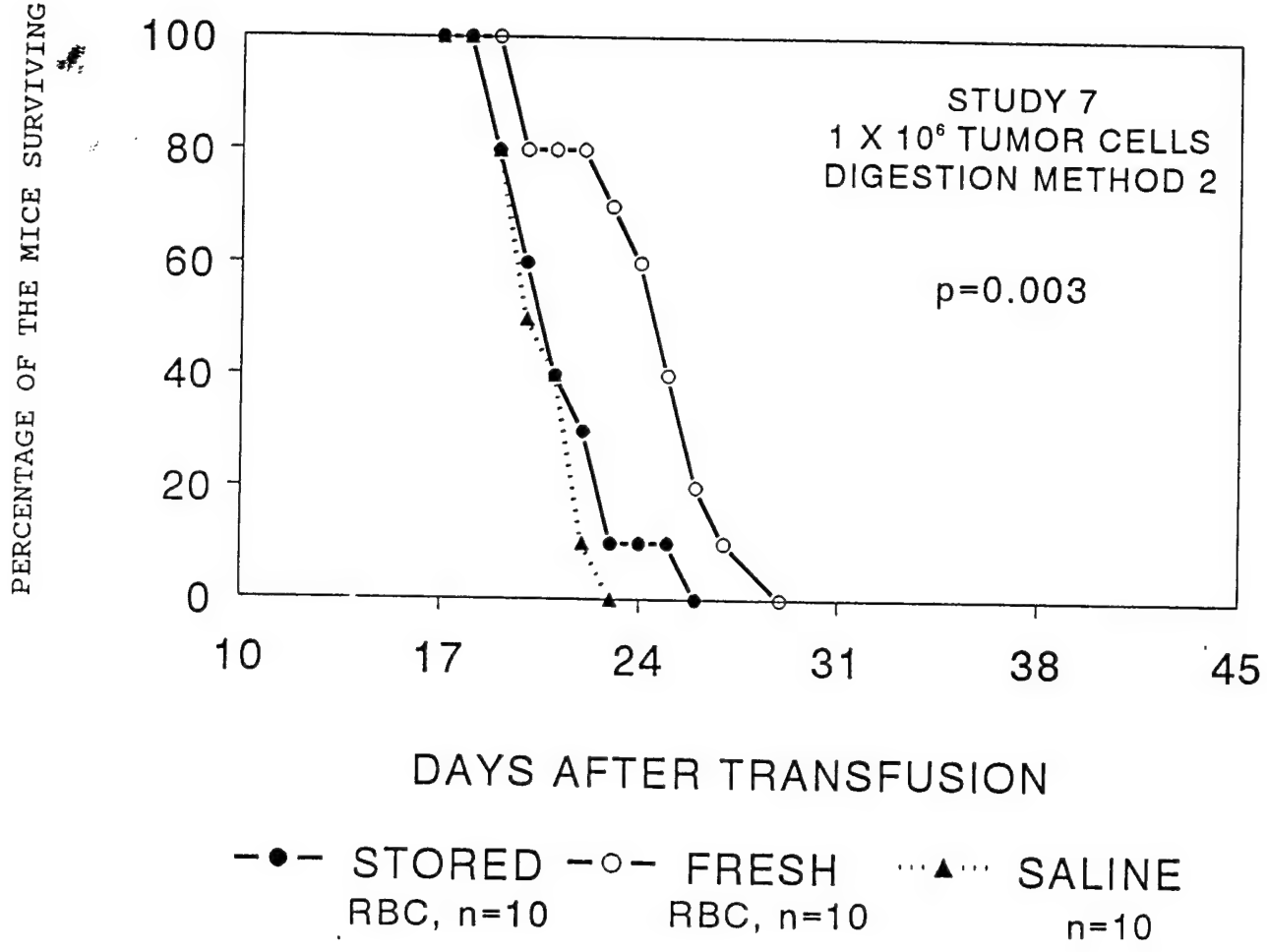


FIGURE 8

NUMBER OF METASTATIC FOCI IN THE LUNGS FOLLOWING THE INFUSION OF 2, 5, 10 AND 20 x 10^6 TUMOR CELLS AT THE TIME OF THE DEATH OF THE MICE IN THREE STUDIES

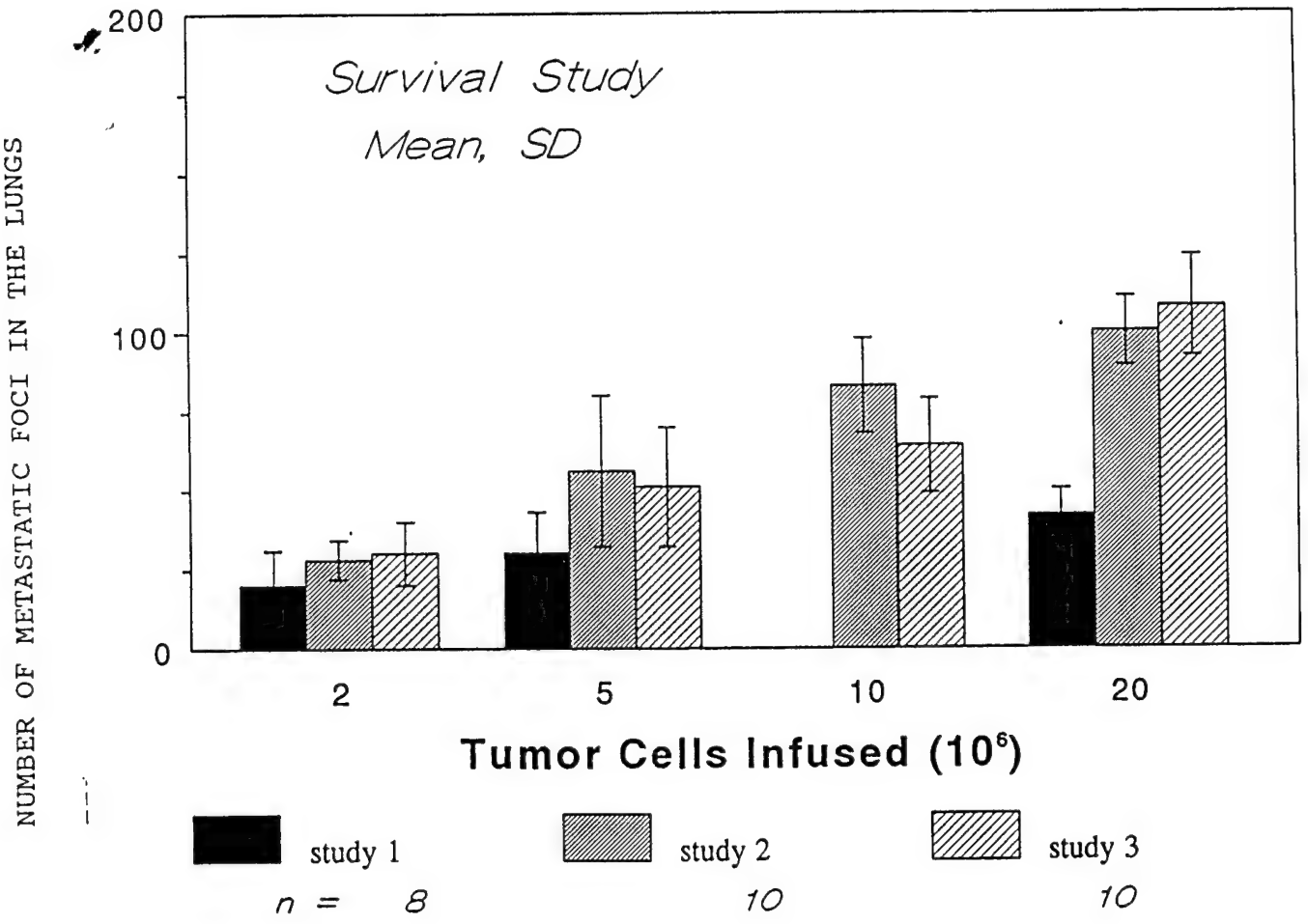


FIGURE 9

THE MEAN DIAMETER OF THE FIVE LARGEST METASTASES IN THE LUNGS OF MICE INFUSED WITH 2,5, 10 AND 20 X 106 TUMOR CELLS AT THE TIME OF THE DEATH OF THE MICE IN THREE STUDIES

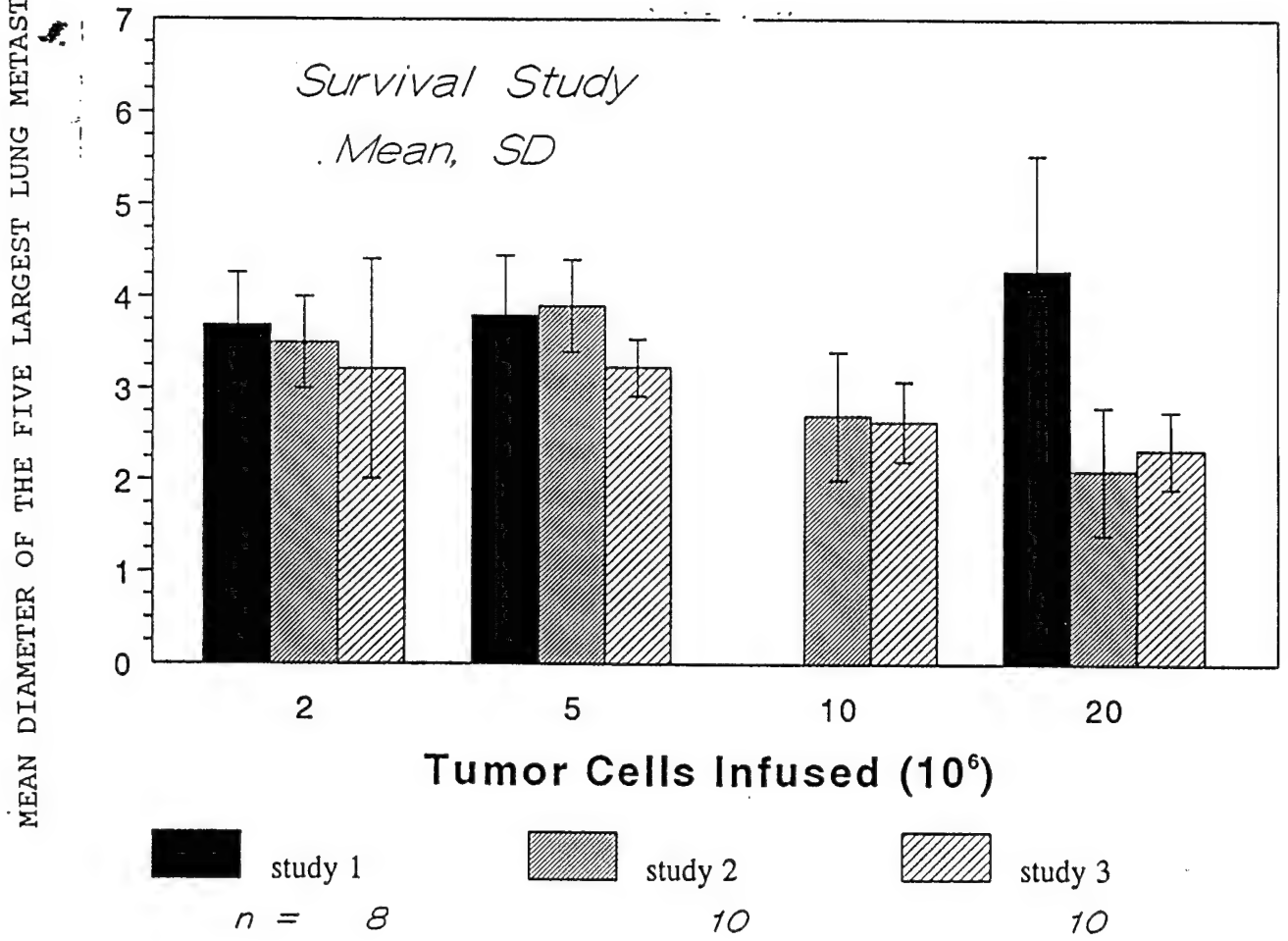


FIGURE 10

NUMBER OF METASTATIC FOCI IN THE LIVER FOLLOWING INFUSION OF 2, 5, 10 AND 20 X 10^6 TUMOR CELLS AT THE TIME OF DEATH OF THE MICE IN THREE STUDIES

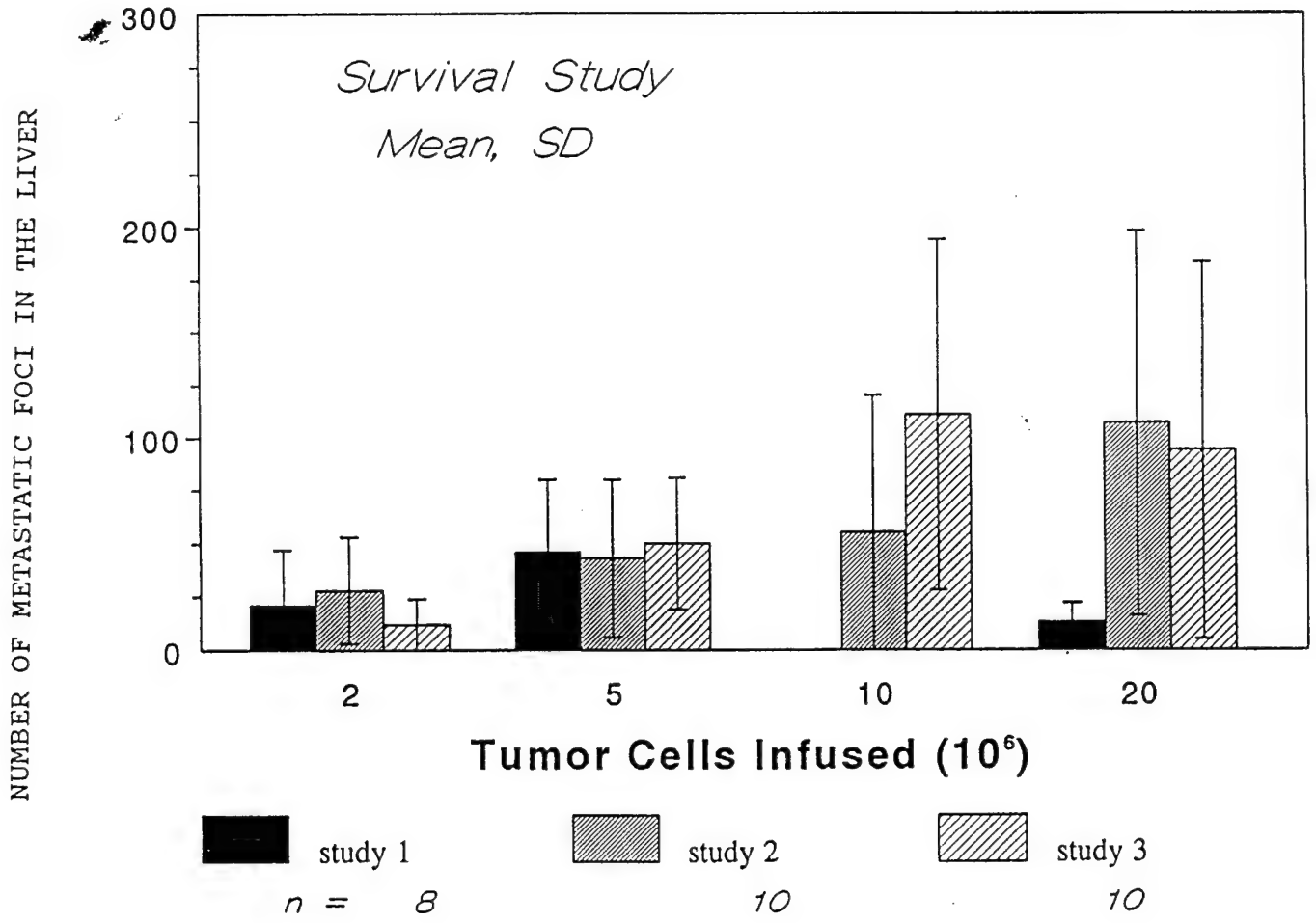


FIGURE 11

THE ACCUMULATION OF 125-IUDR IN THE LUNGS MEASURED AT THE TIME OF SACRIFICE 9 OR 12 DAYS FOLLOWING INFUSION OF 0, 2, 5, 10 AND 20 X 10^6 TUMOR CELLS

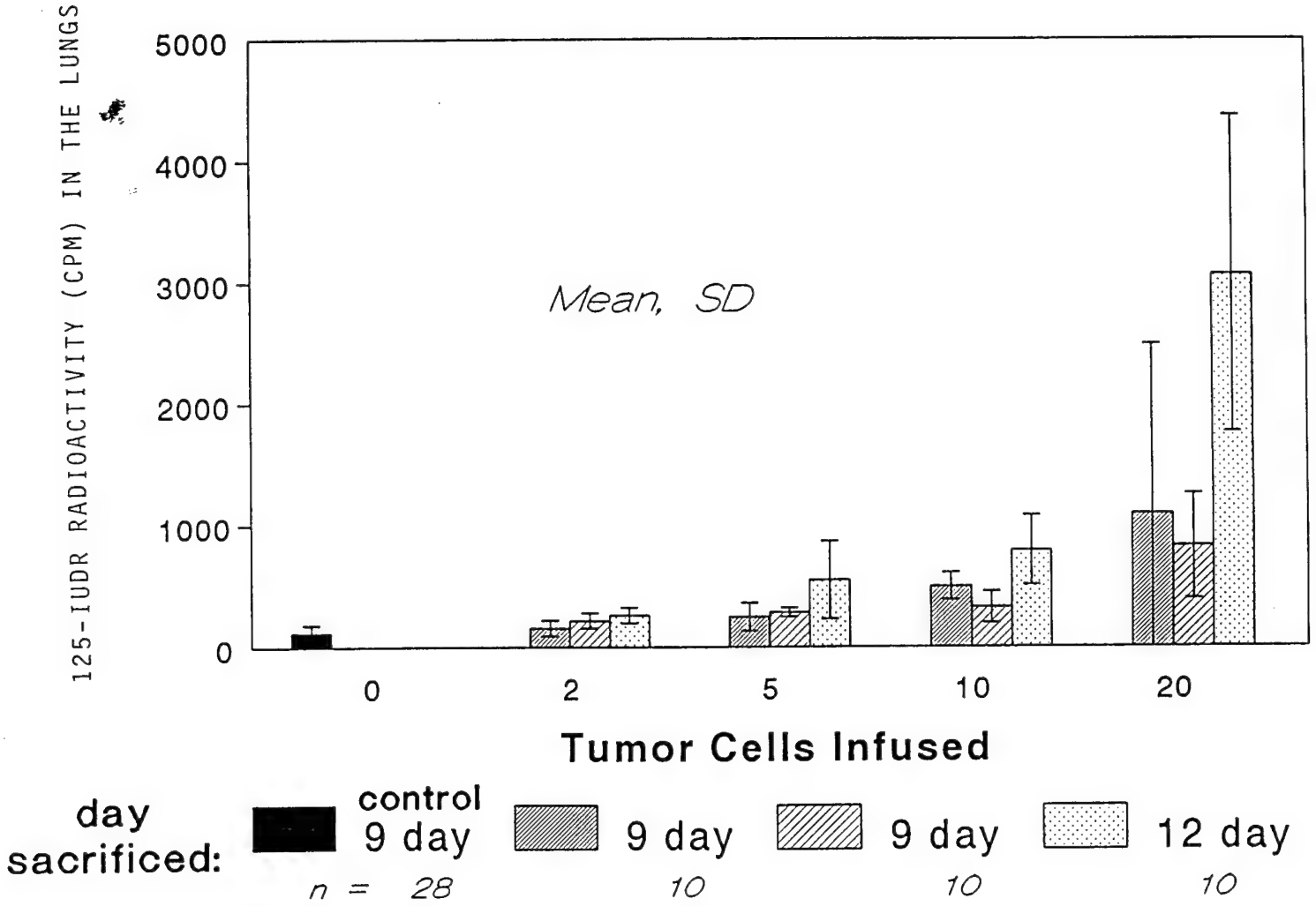


FIGURE 12

THE ACCUMULATION OF 125-IUDR IN THE LIVER MEASURED AT THE TIME OF SACRIFICE 9 OR 12 DAYS FOLLOWING INFUSION OF 0, 2, 5, 10 AND 20 X 10^6 TUMOR CELLS

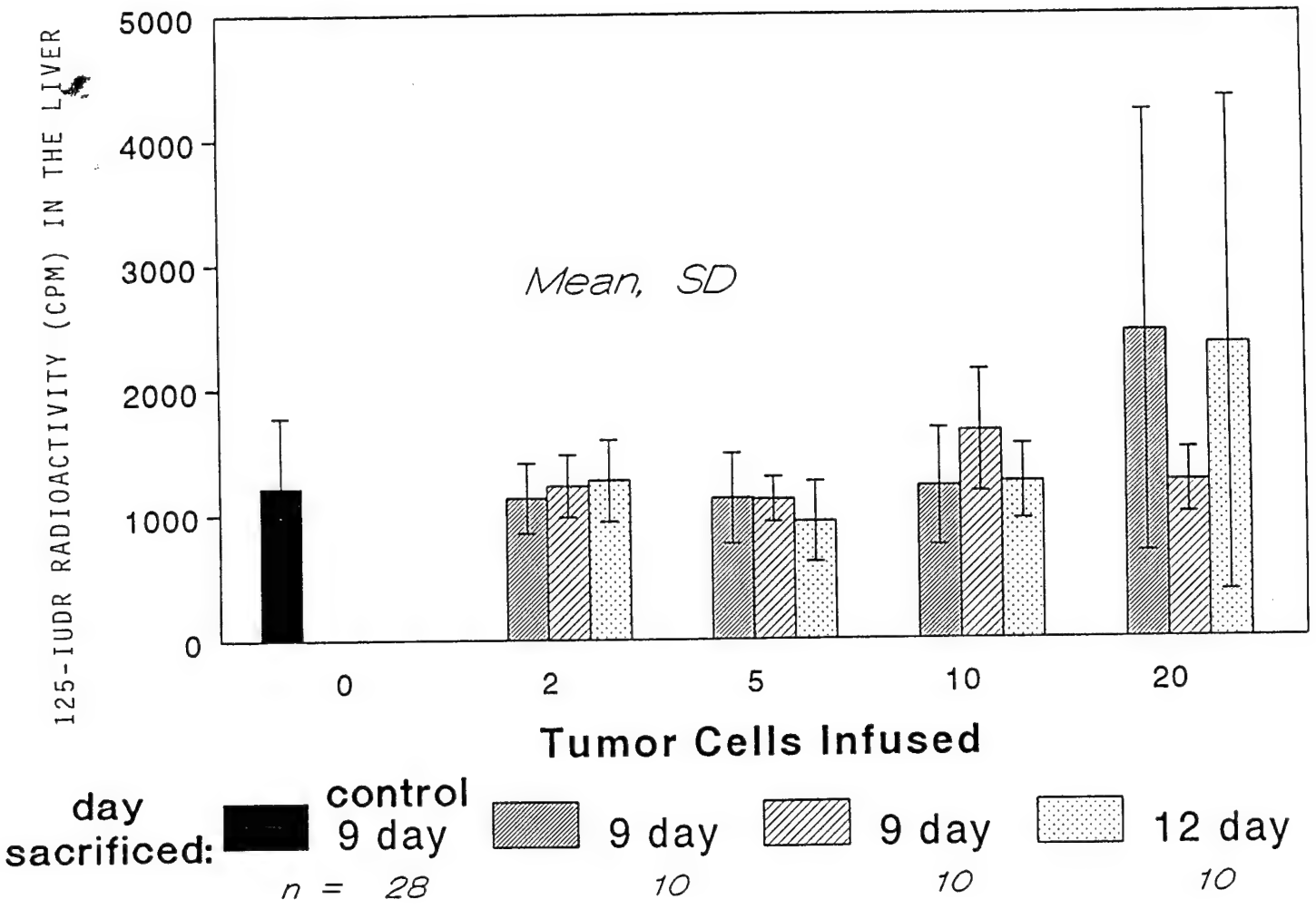
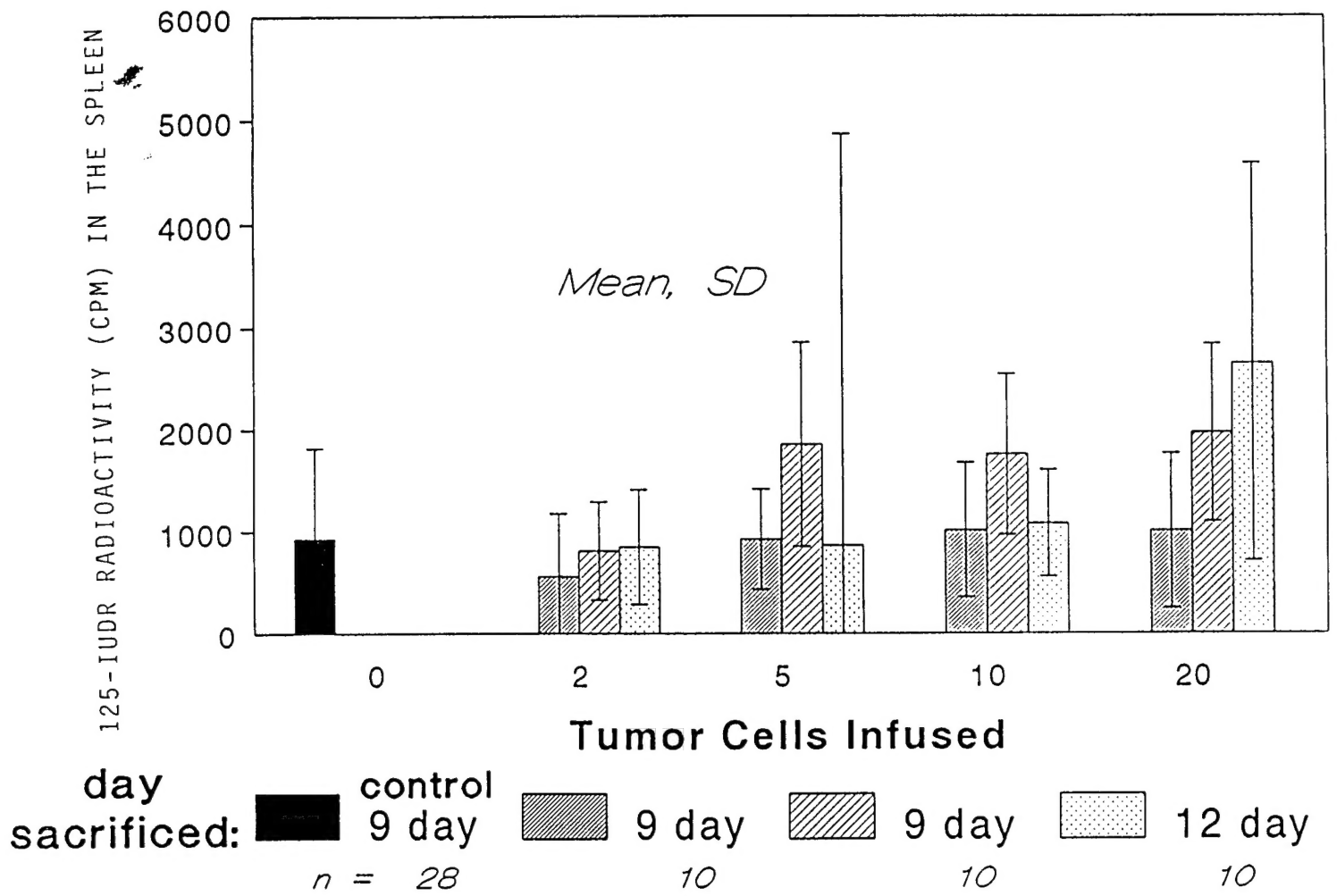


FIGURE 13

THE ACCUMULATION OF 125-IUDR IN THE SPLEEN MEASURED AT THE TIME OF SACRIFICE 9 OR 12 DAYS FOLLOWING INFUSION OF 0, 2, 5, 10 AND 20 X 10^6 TUMOR CELLS



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